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THE COLOURS OF FEATHERS AND
THEIR STRUCTURAL CAUSES IN
VARIETIES OF THE BUDGERIGAR,
MELOPSITTACUS UNDULATUS (SHAW)

by

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THESIS

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INTRODUCTION.

Several papers on the microscopical structure and the pigmentation of the feathers of the Budgerigar have already been published. This bird which is so easily bred and which, under selection, has developed a great number of varieties in two or three decades and practically from the beginning under scientific control, is an object of intense interest for the geneticists. Its varieties differ predominantly by their shades of colour. The subjective impression of a colour, however, is not a criterion sufficiently reliable for distinguishing the varieties. Partly for this reason, but chiefly in order to establish the effects of the genetical factors on both structure and pigment, the researchers included the microscopical examination of feathers and feather-germs in their field of work.

Nevertheless, further research along these lines has been found desirable, chiefly for two reasons. First, since the publication of the papers referred to, a number of new varieties has appeared with shades of colour very different from those known hitherto. These shades suggest that there may be very interesting changes in the structural and pigmentary factors which are responsible for the impression of colour. Secondly, these authors did not undertake a histological investigation of the feathers and feather-germs which could be considered exhaustive from a purely morphological point of view, as they were chiefly

concerned with genetical problems. Moreover, these papers reveal differences in the histological interpretation of the examined material.

The aim of the present investigations, therefore, is to study the coloration of the Budgerigar on an entirely morphological basis, not only the varieties which have originated in the last years, but also a large number of facts concerning the wild-type and the varieties already examined by others.

Thus, I gladly accepted the suggestion kindly made to me by Professor James Ritchie and Professor F. A. E. Crew to devote myself to the subject outlined above. Professor Ritchie also enabled me to work in the Department of Zoology at the University of Edinburgh and put all the necessary requisites at my disposal. Professor Crew provided me with the material for the investigations and gave me the possibility to keep the living birds in the aviaries of the Institute of Animal Genetics. I am grateful to them for their assistance and advice, and also to Professor Charles H. O'Donoghue and Dr. Fabius Gross for their interest in these investigations.

MATERIAL AND METHOD.

For examination, I have used fully developed feathers of different regions, partly taken from preserved specimens which had been eviscerated, impregnated with 40% formaline and dried, partly plucked from living birds. Further, a great number of feather-germs of different stages, taken from living birds, has been examined.

For examination in intact state, the feathers have been mounted in Dammar gum and examined in reflected as well as in transmitted light.

For cutting with the microtome, the fully developed feathers have been directly imbedded either in overheated hard paraffin wax or in Reichensperger's mixture (100 parts of hard paraffin wax heated with 1 part of India rubber), without the use of any intermedia before imbedding. According to my experience, a very slow cooling of the imbedded objects gave much more satisfactory results than the quick one, recommended e.g. by Spoettel (1914).

Sections of 10 to 5 μ were made with a Cambridge rocking microtome, and the majority of these were stained, partly with a saturated solution of Congo red, partly with a saturated solution of Eosine, both in 96% methylated spirit (F). The staining with Congo

F) All spirit used as intermedia or dissolving media was denaturated with methyl alcohol. Instead of absolute alcohol, in all cases terpeneol has been used as an intermedium. The wax was removed from the sections with xylol.

red is, when a neutral mountant is used, much more durable than that with Eosine, but it requires a much longer exposure of the sections to the dye. Both dyes stain all keratin structures equally, but they make the boundaries between the layers and between the cells fairly visible.

Some sections were stained with Gentian violet, according to the direction given by Romeis (Peterfi, 1928, p. 918), except that instead of 2 minutes, I found an exposure of at least 2 or 3 hours to the stain indispensable. This method makes only certain portions of the keratin structure visible, a selective action which I have found of some advantage.

For routine examination, sections were mounted in Dammar gum without staining. Finally, in some sections the imbedding wax has been removed and the sections have been covered in a dry state, with the mountant used only at the corners of the cover-glass. These objects show the true colour of the cloudy medium in reflected as well as in transmitted light.

The feather-germs have been fixed mostly in Dubosq-Brésil's mixture, a few in Carnoy's mixture. While the first-named method gave fairly good results, provided the fixation was not extended over a few weeks, Carnoy's fluid caused in all cases bad shrinkages and a rather indistinct staining. On the other hand, the last-named method made the stages of mitosis more conspicuous in the stained section.

As a last intermedium before using paraffin wax, I used in some cases xylol, in others benzol, methylbenzoate, chloroform, or cedarwood-oil, all of them

with nearly the same results. According to the experiments I have made with feather-germs, there is no advantage in keeping these objects in the paraffin-bath longer than for about 48 hours.

For imbedding the feather-germs I have used either Reichensperger's mixture or pure overheated hard wax. The former gave better coherent serial sections. (F) Besides transverse sections, longitudinal ones have been made of some germs, also 5 μ thick. The trouble, caused by the dragging out of the object by the microtome knife, leaving only the ring of the sheath inside the wax film, has been complained of by many authors, e.g. Strong (1902). When using recently prepared blocks, I also suffered from this inconvenience, but, on the other hand, when cutting blocks some months old, I got nearly complete series.

The sections have been stained either with Haematoxyline (Delafield or Ehrlich) and Eosine, or, in the most cases, with Azan staining, according to Mallory and Heidenhain's method (Peterfi, 1928, p.926).

The usual Haematoxyline-Eosine method has the advantage of being carried out very quickly. It gives a fairly good differentiation of the stages of keratinization, particularly after a slight overstaining

(F) I have not been able to try 'Espinasse's imbedding medium (Bolles Lee's "Microtomist's Vade Mecum", tenth edition, p.96), consisting of Ceresine, candle-wax, and soft paraffin-wax, not having seen his publication before concluding my work.

with haematoxyline: then the non-keratinized cytoplasm shows a more or less lilac-reddish colour, while the keratin structures appear bright red. This result has been indicated already by Strong (1902). The Eosine staining, however, has the well-known disadvantage of being not very durable.

Azan staining has been used with some modifications: the impregnation with the solution of phosphotungstic acid has been carried through for at least 4 hours, and the counterstaining extended up to 12 or 14 hours, otherwise the results would have been very unsatisfactory. After fixation in Dubosq's fluid, which was not prolonged for a very long time, the staining made the not yet keratinized epidermal cytoplasm appear light violet blue, while the different stages of keratinization accepted shades from yellow up to bright red. After longer fixation, the epidermal cytoplasm did not take the counterstain very well: it remained pinkish and therefore did not give a very good distinction of the keratinizing structures.

The terminology used in the present paper to indicate the different positions of structures within the transverse section of a barb of the feather, as well as for the corresponding directions, is as follows: -

Structures situated near the
centre of the whole barb: "central"
(or "axial").

Structures situated near the
surface of the whole barb: "peripheral".

Structures situated near the
centre of a single medullary cell: "internal".

Structures situated near the
surface of a single medullary cell: "external".

Structures situated near that
portion of the surface of the barb
which in the plumage of the intact
bird is directed towards the observer (F):
"ectal".

Structures situated near that
portion of the surface of the barb
which is, under the same conditions,
directed towards the skin of the bird (F):
"ental".

F) In most papers dealing with the histology of
bird-feathers there are used for both of these
positions or directions the terms "dorsal" and
"ventral". But, when dealing with the feathers
of the under surface of a bird, these terms
could give rise to misunderstanding. Therefore,
I replace them here by terms used in the topo-
graphy of hairs.

CRITICAL REMARKS AND SUPPLEMENTS TO THE DESCRIPTION OF THE WILD-TYPE AND THE VARIETIES EXAMINED BY PREVIOUS WORKERS.

a) Physical principles of the structural blue and green colour.

The structure of the green feathers of the Budgerigar was first investigated by Kniesche (1914). This author bases his conclusions on Haecker and Meyer's examination (1902) of feathers with non-metallic blue colour, and he extends his investigation over a large number of green feathers of different birds, among them those of the wild-type of the Budgerigar.

According to Haecker and Meyer, non-metallic blue colour is caused by an arrangement of two different optical media in the barb of the feather: a cloudy layer beneath which is situated a dark one. The physical explanation the authors give for the colour-producing effect of this arrangement is based on Lord Rayleigh's statements.

This physicist explains a cloudy medium as consisting of an arrangement of very small particles of two transparent media, each of a different refractive index. The diameter of these particles must be smaller than the wavelengths within the spectrum of white light. Under such condition, due to the fact that the intensity of reflected light is inversely proportional to the fourth power of the wavelength of

this light, the shorter waves dominate among those reflected by this medium, while the remainder will be transmitted through it. The dark medium underneath absorbs these transmitted longer waves.

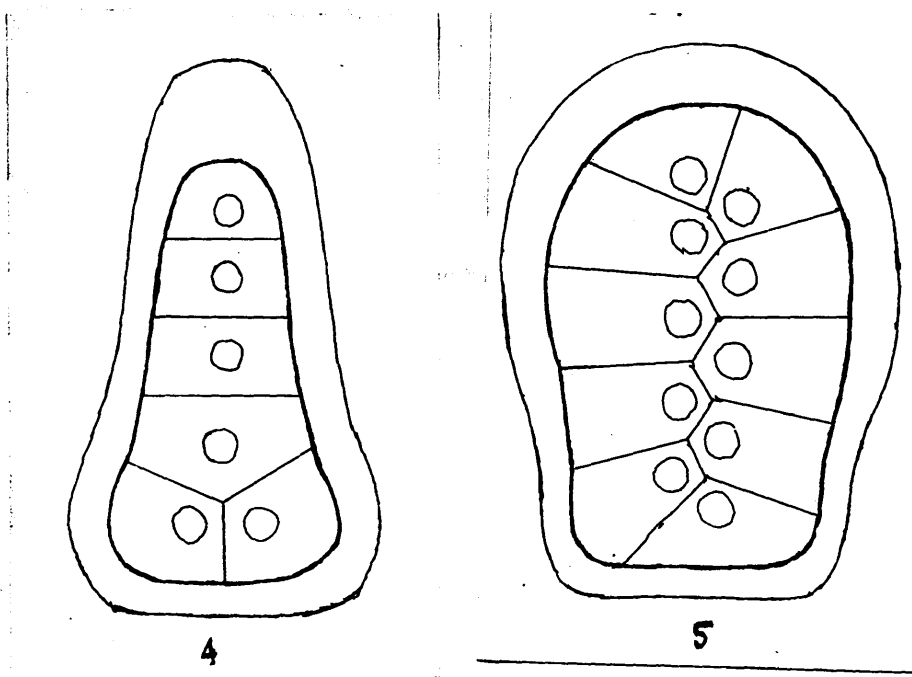
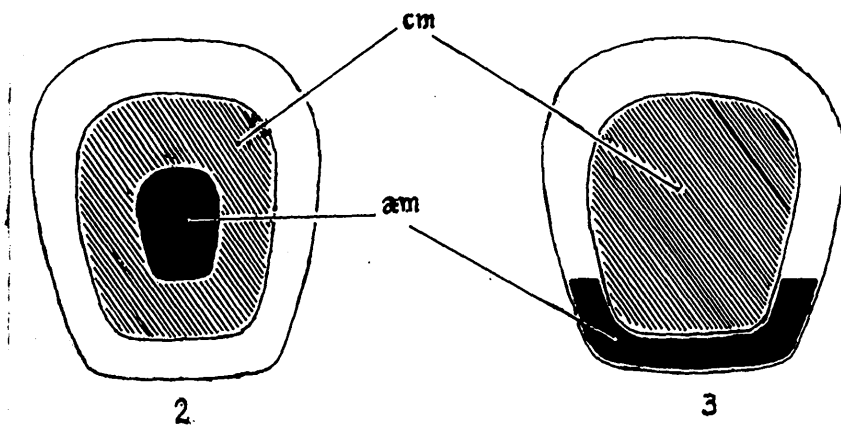
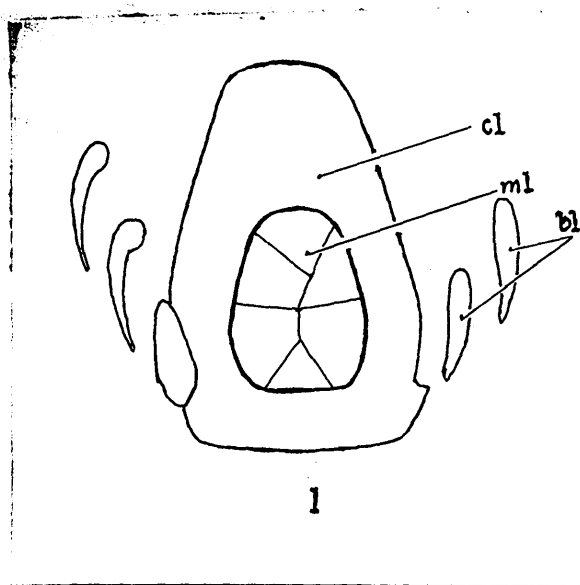
In reflected light, the effect of this action is a subjective impression of a colour shade between greenish blue and violet, depending on the dimensions of the particles within the cloudy medium. In transmitted light this cloudy medium produces an impression corresponding to the transmitted longer wavelengths, i.e. of a shade between orange and yellow, depending again on the size of the particles.

According to Kniesche, in these cases of structural colour, green shades are produced by the presence of a yellow pigment in the ectal transparent portion of the layer above the cloudy medium. In blue feathers this portion is practically colourless.

b) The morphology of the
structural blue and
green feathers in
general.

As mentioned above, the two media which are the principal factors causing a non-metallic structural blue or green colour are restricted to the feather-barbs. The barbules, on the other hand, seem to partake only in a less important way in the fixation of a special shade of these colours, a point which will be discussed later. (F)

F) In only one case, namely the Papuan Pigeon, genus Goura, the barbules contain both the cloudy and the absorbing dark media and, thus, produce



- 1) Diagram of the transverse section of a barb.
 - cl cortical layer,
 - ml medullary layer, showing cell-boundaries,
 - bl barbules.
- 2, 3) Diagrams of the two chief possibilities of forming an absorbing medium in structural green or blue feathers, - either by the axial portions of the medulla (Fig. 2),
or
the ental portions of the cortex (Fig. 3).
 - cm cloudy medium,
 - am absorbing medium.
- 4, 5) Diagrams of arrangement of the medullary cells in the green feathers of the Budgerigar, - either uniserial (Fig. 4), or alternating (Fig. 5).

In general, the transverse section of a barb (Fig. 1) shows a cortical layer which surrounds a medullary one. In the blue or green feathers as well as in many other cases, at least the ectal portions of the cortical layer are transparent. In the green feathers these portions contain, as already indicated, an apparently diffuse yellow lipochrome pigment which produces together with the structural blue colour a compound green one.

In the fully keratinized feather the cortex appears as a nearly homogeneous substance in which cell-boundaries are not very easily distinguished. The medulla, on the other hand, shows a more complicated structure in which, partly at least, are to be distinguished boundaries which separate large cells.

The cloudy medium is always provided by the medulla or, at least, by its more peripheral or ectal portions. The dark medium, on the other hand, consists, as can be seen from the numerous semi-diagrammatic illustrations given by Kniesche and from the figures 2 and 3 in the present paper, either

A) of some more or less continuous medullary portions which form an axis of the barb as a whole, or

B) of the ental portions of the cortex.

The feathers of the Sudgerigar belong to the first of these two chief types. The black impression of this medium is always brought about by the presence

∕. the blue colour itself, according to Spoettel (1914).

of distinct granules which are impregnated with melanin pigment.

Concerning the histological structure of the two media, it is quite obvious that in the instance described under B there are separate cloudy cells and others whose exclusive function is to form the absorbing dark background, both of them belonging to different structural elements of the feather.

In the case A in which both media are found within the medulla, Haecker and Meyer as well as Kniesche speak in general of a "layer of cloudy cells" and of one formed by "dark cells".

In a previous publication (1890) Haecker, however, describes the structure of the black and blue barred feathers of Garrulus glandarius Linn. Within the black bars he has found a colourless ectal portion of the cortex, with the medullary portions immediately beneath it intensely melanized. Towards the blue bar, the pigment in these portions of the medulla "gradually recedes towards the posterior [i.e. ental] walls of the cells forming these medullary portions": then these bare cell-portions turn out to operate as a cloudy medium.

This instance of combining both media in one and the same cell was not further referred to either in Haecker's later publication or in the papers of the other authors. Later discussions will deal with the question whether in the feathers of the Budgerigar each of both media is composed of separate cells, or whether both of them are provided by the same cell.

In the "cloudy cells" Haecker and Meyer have established a system of delicate air-filled channels

which perforate the keratinous substance. As the transparent keratin has a refractive index which is higher than that of the air, and the diameter of the channels is said to be smaller than the wavelength of the light, this arrangement is in accordance with Lord Rayleigh's definition of a cloudy medium.

Kniesche gives further particulars of this canalicular structure. The central part of each "cell" is occupied by a cavity which is of a more spherical shape when it is small and the keratinous wall thick. Otherwise the wall is thinner and the vacuole is larger, with its outlines following more or less those of the external surface of the cloudy cell, the vacuole being thus square or polygonal. Beginning from this cavity, a great number of the channels mentioned above perforate the keratinous wall in a radial fashion. Provided the latter is thin, then the channels go straight through the wall up to its external surface. If the wall is comparatively thicker, then the channels are rectilinear only for a short distance from the vacuole: in a more external zone of the wall they become entangled or disappear among irregular clefts within the horny substance, while in the most superficial part of the wall, there appear again straight channels which reach the external surface in radial arrangement, as in the first case.

According to Kniesche's hypothesis, this system of channels and the vacuole might be the effect of gradual keratinization under the control of surface tension. At first, inside the cell a gas-bubble originates in the course of chemical changes. Then, from the outer surface of the wall on the one

hand and from that of the gas-bubble on the other, the coagulation of the plasma takes place with a minimal increasing of the surface of the solid substance, forming thus straight cylindrical channels. In the middle between the two surfaces, the progress is less controlled, and thus irregular clefts are formed.

Kniesche examined only full-grown feathers and made the delicate channels visible by impregnating the barbs with an alcoholic solution of shellac. He emphasizes, however, that he has not been successful with this method in every case in which he has established a cloudy medium by its optical effect.

a) The histology of the green feather
of the wild-type of the Budgerigar.

A transverse section of 10μ , made of a barb of an abdominal feather of the wild-type, is figured by Kniesche. It shows the cloudy medium divided by cell-boundaries in a ring-shaped series of more than twenty compartments, each of them showing in its centre a circular vacuole. The melanin axis is distinctly demarcated against the cloudy zone so that there is no doubt that the author expects this demarcation to coincide with cell-boundaries. The melanin granules in this axis are also distinctly represented, in comparison with the other figures on the same plate. In the centre of the axial space the granules are arranged in definite circular spots.

Steiner (1932), in his profound genetical paper, devotes a chapter to the histology of the feathers and feather-germs. He confirms Kniesche's results. In

a transverse section of 3μ , or an abdominal feather of the budgerigar, he found below the cortex "an unicellular layer of polygonal canalicular cells

A two- or threefold layer of spheric medullary cells forms the centre of the space". He emphasizes that he was not able to make the channels visible with the method recommended by Kniesche, but he could establish them by means of a dark-ground condenser. He describes the channels as being irregularly entangled. This author does not mention any structure of the shape of vacuoles. The "spheric medullary cells" correspond, without doubt, with the circular spots in the medulla which are to be seen in Kniesche's figure.

Quite different observations have been made by Kawamura (1935) who examined feathers and feather-germs of the same region of the wild-type as has been dealt with by the above-mentioned authors. Kawamura's illustration shows the distinctly axial arrangement of the media, but its outlines are somewhat triangular, and not oval, as in Kniesche's paper. The medulla consists in its ectal half of a small number of very broad cells, each of them occupying the whole width of the medulla. Thus, the arrangement of the cells within this portion of the medulla is a strictly serial one. In the ental half of the medulla, this arrangement seems to be somewhat disturbed: there are some smaller cells in greater number. Along the median region of the medulla each cell shows a large circular vacuole, these being arranged therefore also in a vertical series in the ectal half of the medulla. Around each of these vacuoles, ovoid pigment granules are arranged in a rather regular way, with their longitudinal axes

directed mostly radially. The groups of these granules reach the ectal and the ental boundaries of each of the cells, but they leave large cell-portions adjacent to the cortex bare. In such a way, similar to that described in Haecker's earlier publication (see above, p. 11), each medullary cell contains portions of the cloudy as well as of the absorbing medium. By the arrangement of these portions within each single cell as well as of the whole cells within the medulla, the latter cooperate in forming a coherent absorbing axis surrounded by a ring-shaped cloudy zone.

Compared with Kniesche's figure, that given by Kawamura shows a much broader cloudy zone in proportion. The last-named author emphasizes that there is no vacuole in the single portions composing the cloudy ring, i.e. on the spots where vacuoles are drawn in Kniesche's figure.

In his examination of the feather-germ, Kawamura proves that the large spheric cavities inside the absorbing axis have replaced the nuclei of the medullary cells during keratinization. During the differentiation of the germ into the full-grown feather, the successive stages show a considerable growth of the medullary cell-bodies, directed peripherally. During this growth, the nuclei remain in the median region of the barb.

In order to check the differences in the statements made by the authors referred to, I examined feathers of the abdomen of different specimens of the wild-type in different ontogenetic stages. Concerning the demarcation of the single medullary cells, Kawamura's state-

ment seems to me fully confirmed: the arrangement of the two media within the single cell is to be seen distinctly even in unstained sections of 10μ , of the full-grown feathers.

I have observed triangular outlines of the sections in many feathers of this region, while others, even of the same bird, show a more longitudinally rectangular or oval shape. In the latter case, the serial arrangement found in the ectal parts of the medulla is mostly replaced by another one, in which the cell-bodies do not extend from wall to wall of the cortex but touch only one side. In this case, the nuclei are not arranged in one straight vertical line but in a more or less alternating fashion (Fig. 4 and 5). Later I shall try to explain the causes which could be responsible for the one or the other arrangement.

In many of the stained sections, a number of portions of the cloudy ring, each separated from the other by the cell-boundary, shows a very distinct round vacuole in its centre (in Fig. 8). On the other hand, none of the sections examined by me showed such a large number of cell-boundaries and vacuoles along the cloudy ring, as are shown in Kniesche's figure.

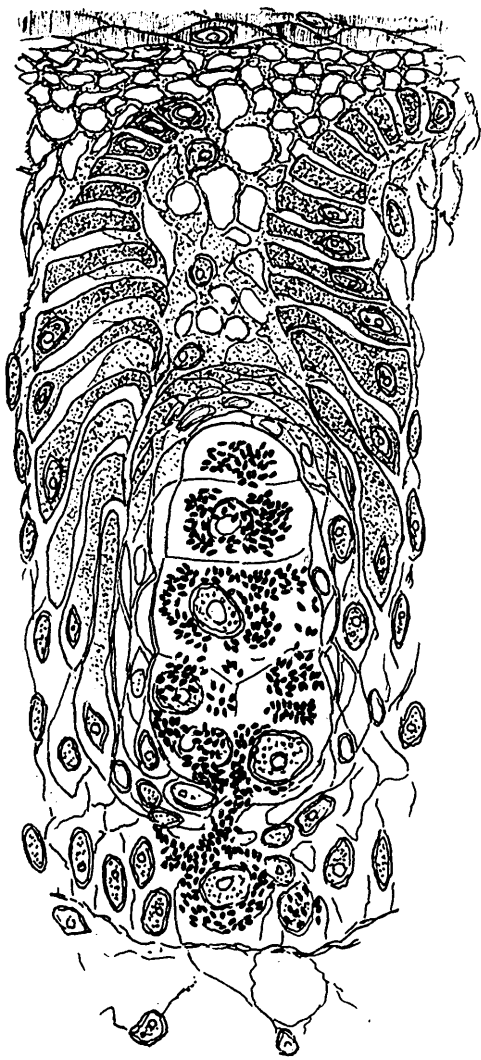
By examining serial sections of feather-germs of this region of the wild-type, I have been able to confirm Kawamura's statement concerning the extreme peripheral growth of the bodies of the future medullary cells, as well as concerning the nuclear origin of the large vacuoles within the axis. In favour of the demarcation of the medullary cells given by this author,

and against that given in the other papers mentioned, except for the earliest one published by Haecker (1890), are the facts that

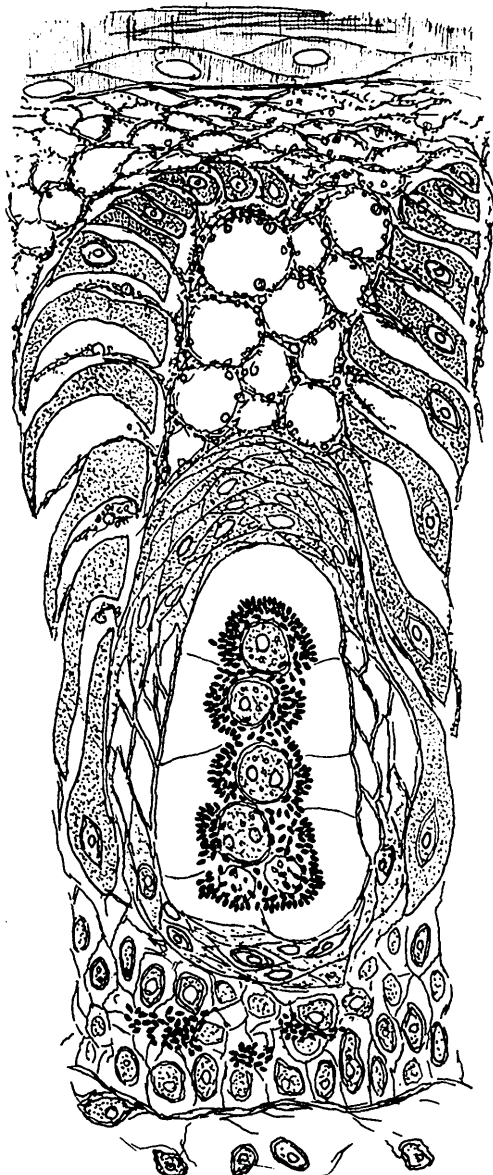
- 1) in no stage of development from beginning of peripheral growth of the single medullary cell-bodies, can any nuclei be made out in the medulla, except for those inside the axis, and that
- 2) in the sections of the feather-germs of the corresponding regions of albinos, "yellow" birds, etc., the reduction of pigment allows a better survey of the structures. In these cases, the cell-boundaries beginning at the inner wall of the cortex can be traced up to the centre of the medulla (Fig. 52, 57-59). Nowhere can cell-boundaries be traced, which could separate the peripheral cloudy zone from the central portions.

Thus, the round pigmented spots inside the axis seen in Kniesche's illustration, and Steiner's "spheric medullary cells", cannot be anything else than the axial nuclear vacuoles with the pigment granules arranged around them, seen in more or less tangential optical sections.

As far as I have been able to establish from the examination of the feather-germs, the "periaxial" vacuoles seen in the centre of the single cloudy cell-portions do not seem to be preformed by any special cytological structure. In the sections corresponding with more advanced stages of the differentiation of the feather, there is no trace of them to be seen be-



6



7

- 6, 7) Two successive developmental stages of a barb with barbules in the germ of a green feather taken from the abdomen of the wild-type.

In Fig. 6, the ental portions of the cortex are not yet well-defined from the residual cells, and the peripheral growth of the unpigmented portions of the medullary cells is only indicated in the ectal half of the medulla.

In Fig. 7, the outlines of the cortex are indicated also in the ental portions of the section; the pigmented axis is well-defined throughout the medulla, and the ectal residual cells show many distinct spheric centres of keratinization and large nuclear vacuoles.

(Fig. 6, 7: magnification $\times 1300$.)

fore the axial nuclei have disappeared, and before the centres of the cloudy portions are fully keratinized, according to their staining.

In the sections of the germs of the green feathers of the wild-type, differentiation is shown to take place in a way corresponding in general with that described by Strong (1902) for some other types of birds. A fact which seems to be slightly indicated in the object figured by him (Plate 4, Fig. 21), but which is not mentioned in his papers is the following. After the differentiation of the barboles that of the barbs begins in such a way that at first the ectal portions of the cortex become outlined. The cells of these portions are already flattened and their nuclei partly obliterated, while the ental portions of the cortex as well as of the medulla still show typical epithelial structure and are not yet demarcated from the future ental residual cells (Fig. 6).

At this stage, however, the ectal medullary cells already show the increased size of their cytoplasm and the arrangement outlined in Fig. 4. The latter is a consequence of the cellular arrangement even in very early stages of differentiation of the germ: in these stages, only one vertical layer of cells gives rise to the ectal part of the future medulla, within the ectal half of the tissue preforming the barb. Meanwhile in the ental parts cell-divisions horizontal in direction, or more irregular, are still to be seen (F').

F') This type of division and arrangement of cells in the future medulla seems to be very common in many groups of birds, in which the more triangular

The arrangement outlined in Fig. 5 is combined with a larger number of cells in the ectal part of the medulla, as may be seen at a glance from the much higher number of axial vacuoles in the section. Apart from the increased number of cells the other conditions may be the same, e.g. the tendency to increase the volume of the cytoplasm. Thus, these cells are forced to fill out a space comparatively much more restricted than in the case represented in Fig. 4. Such a pressure in "vertical" (i.e. ecto-ental) direction might also produce the nearly elliptic arrangement of the nuclear vacuoles in very broad barbs (Fig. 59).

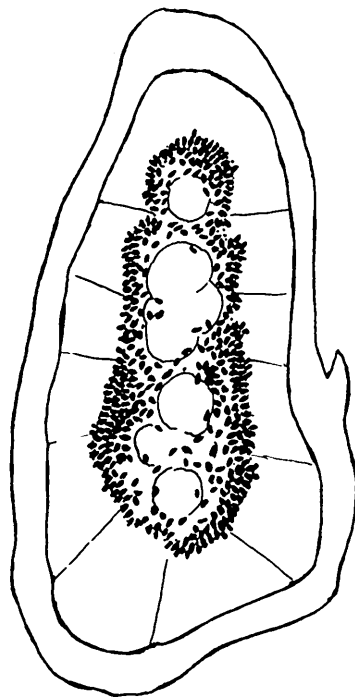
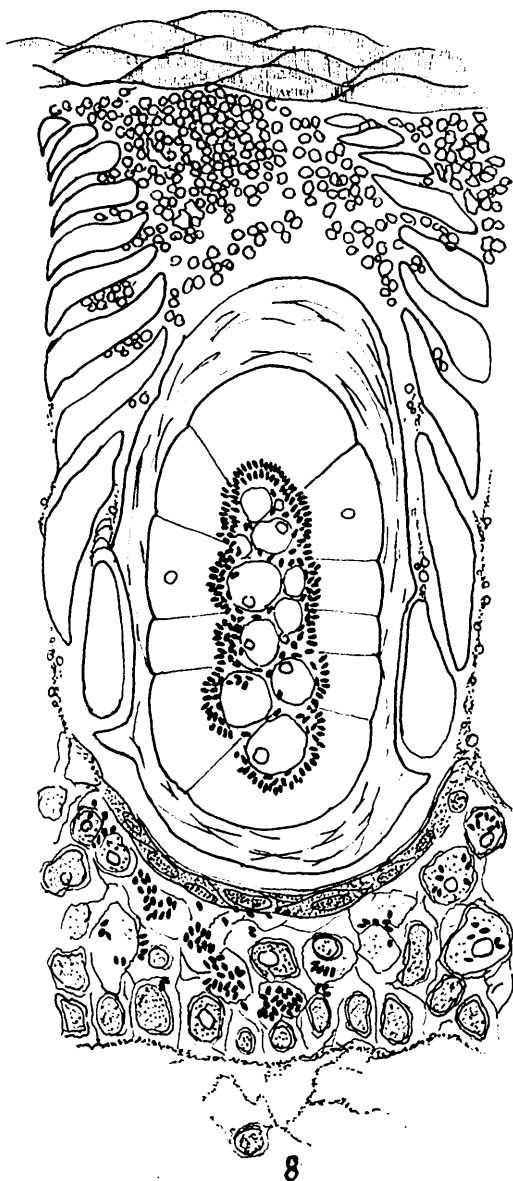
As revealed by staining (p. 6), the first signs of gradual keratinization are to be observed in the barbules, then in the ectal, and from there progressing to the ental portions of the cortex. Within the medullary cells keratinization first appears inside the pigmentary axis around the nuclear vacuoles, in some

./.. or pear-shaped outline of the barb-sections appears again and again, with more or less distinctly uniserial arrangement in the ectal and more irregular one in the ental portion of the medulla. Haecker and Meyer (1902) assume that this type of transverse section is characteristic of Columbiformes and Charadriiformes and therefore conclude that it might be used as a sign of close systematic relation of the birds in which it is to be found. But the wide distribution of this outline and the fact that it is found mostly in a juvenile stage and is replaced often in the course of ontogeny by more or less different types of barbs, - **as in the Budgerigar, - induces me to** consider it a phylogenetically primitive feature.

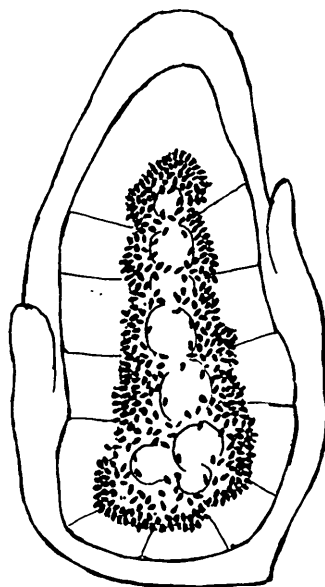
sections also along the inner wall of the cortex. In the wild-type and, as far as I have observed, in all other varieties, except for the "recessive grey" bird (pp. 103 ff.), no distinctly demarked centres of keratinization occur in the medulla. In the barbules and the cortex, on the other hand, there appears always as a first sign of keratinization, a temporary structure which, in the transverse sections, seems to be a granulated one. This granulated appearance, of course, is caused by the longitudinal keratin fibres which are also cut transversally. This seemingly granular structure, however, will be replaced by the apparently homogeneous one, typical of the completed barbules and cortex, before the keratinization of the medulla is completed.

As the sections approach the terminal and highest differentiated portion of the germ, there is noticeable a gradual progress of the staining typical of keratin over the whole body of the medullary cell. This progress is seldom completed before the nucleus is obliterated.

The periaxial vacuoles do not become visible before the almost complete keratinization of the cytoplasm. In accordance with the progress of keratinization within the cell and Kniesche's explanation (pp. 12, 13), the characteristic situation occupied by this vacuole is obvious. It must arise where the least resistance against an accumulation of gas occurs, i.e. in the final remains of still liquid or semi-liquid cytoplasm. Thus, only the form of the cell and the progress of keratinization might be responsible for their situation, and not any cytological structure,



9



10

- 8) Advanced developmental stage of a barb with barbules in the same germ as in Fig. 6, 7.

The barbs and barbules are completely keratinized and the nuclei of the medulla already obliterated, the nucleoli still to be seen in the vacuoles. In the cloudy layer two periaxial vacuoles are to be seen.

The ectal residual cells are nearly fully keratinized and form a dense mass of spheric granules, while the ental ones are separated from the barb by a keratinized membrane formed of flattened cells.

- 9, 10) Wild-type, lower abdomen, sections through barbs towards their tips, of triangular outline.

(Fig. 8 - 10: magnification $\times 1300$.)

as is the case in the formation of the nuclear vacuoles.

In albinos and some other forms a more or less complete circle of many small vacuoles (Fig. 52, 53, 54) is visible round each of the nuclear ones. These "peri-nuclear" vacuoles may also be formed by gas-bubbles which arise where keratinization first takes place. The keratinization of these cell-portions may be a much quicker one than that of the remainder of the cell.

The residual cells which fill the space ectal from the barb and between the two series of barbules show obliteration of their nuclei under formation of vacuoles even when the ental portion of the barb still shows a low degree of differentiation. Later, through the increase of the size of these nuclear vacuoles, the residual tissue has the appearance of a network (in fig. 7), the meshes of which contain large numbers of distinct keratinous spherules. Some of these spherules also occur here and there in the narrow spaces between the single barbules. Still later, when the fully differentiated barbs first become loose on their ectal outlines, vacuoles can no longer be seen in the now completely keratinized ectal residual tissue, which then seems to consist only of a dense mosaic of the spherules (in fig. 8).

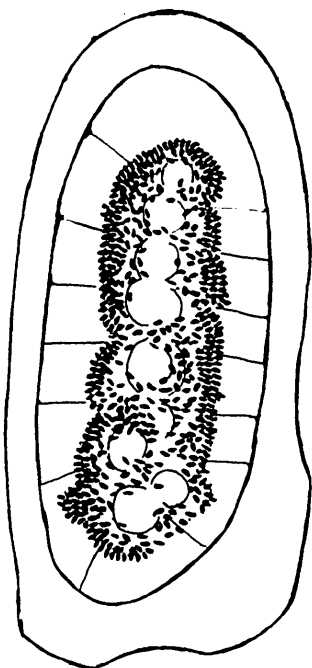
The ental residual cells, together with the layer of cylindrical cells, retain their character of living epithelial tissue, and they do not show any degeneration of nuclei inside their layer. Between these cells and the barbs, loosening takes place much later than on the ectal side. It is preformed by the re-

residual layer which develops a continuous but rather narrow membrane consisting of flattened and keratinized cells. After the withdrawal of the barb, this membrane remains adherent to the residual epithelium (in fig. 8).

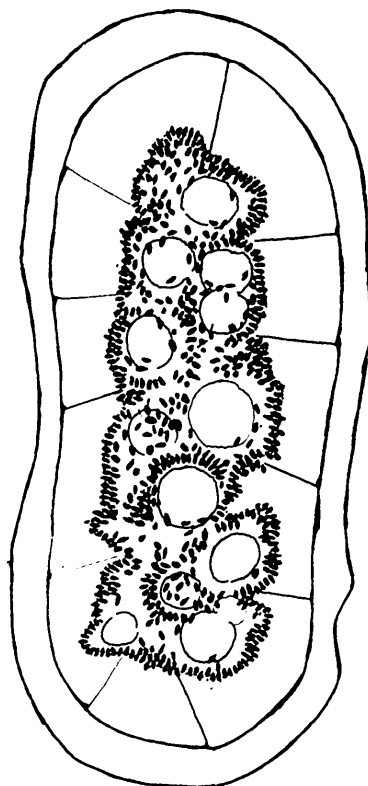
It is remarkable that in the nuclear vacuoles of the medulla of these barbs, after the obliteration of the nuclei, large nucleoli are still present. Schwann (1847) has observed a similar ~~outlasting~~ of the nuclei by the nucleoli in the medullary cells of the rachis of other birds.

d) Differences in the outline of the
barb-sections, and importance of
the barbules for the tint.

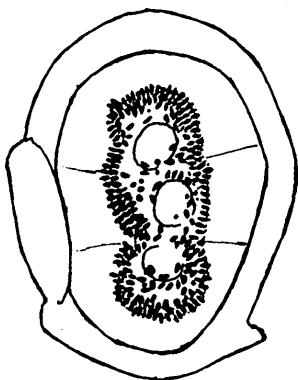
As mentioned on p. 16, I have found among the sections of feathers of the lower abdomen some with distinctly triangular outlines, others more parallelogram-shaped or even oval. In one specimen bred from an imported pair they had triangular outlines (Fig. 9, 10), but others showed broader ectal parts, the uniserial arrangement of the medullary cells being almost entirely replaced by the alternating one. In feathers of the same region of other specimens, sections with oval outline have ~~often been~~ found, in which the ectal parts of each section are slightly broader than the ental ones, as shown in Fig. 12. Even in the same section of a feather or of the terminal part of a germ great variation occurs in these outlines in quite irregular arrangement. In the proximal parts of the barbs of these feathers, however,



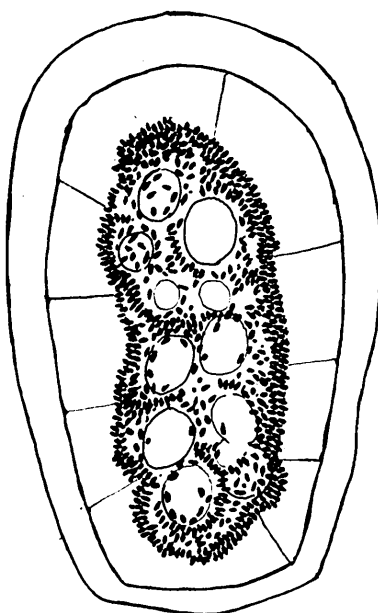
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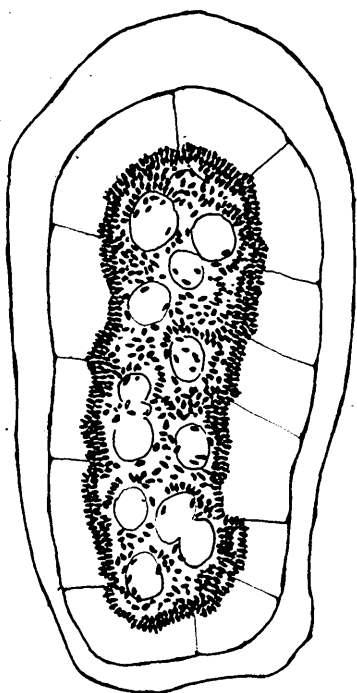
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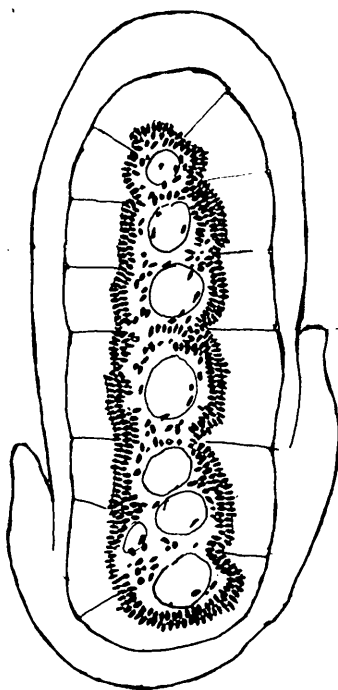
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- 11) Wild-type, lower abdomen (same feather as in Fig. 9, 10), section through a barb towards its base.
- 12) ditto., feather of another specimen, middle part of barb.
- 13) Wild-type, rump, section through the tip of barb.
- 14) ditto., middle part of barb.

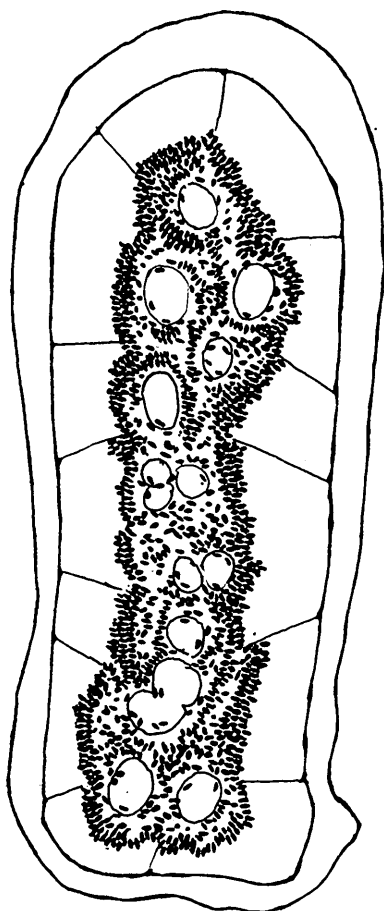
(Fig. 11 - 14: magnification $\times 1300$.)



15



16



17

- 15) Wild-type, rump (same feather as in Fig. 13, 14), section through the middle part of barb.
- 16) ditto., basal part of barb.
- 17) ditto., feather of another specimen, middle part of barb.

(Fig. 15 - 17: magnification $\times 1300$.)

the sections nearly always show greater lateral compression, and are consequently narrower. They have also greater longitudinal diameters than the sections of more distal portions of the same barb (Fig. 11).

In the triangular sections there is an indication of a crest-shaped thickening of the ectal portion of the cortex, a feature mentioned by Kawamura. In this ectal crest the yellow peripheral zone of the cortex is much thicker than in the lateral and ental portions. In this body-region, the barbules are intensely yellow in colour.

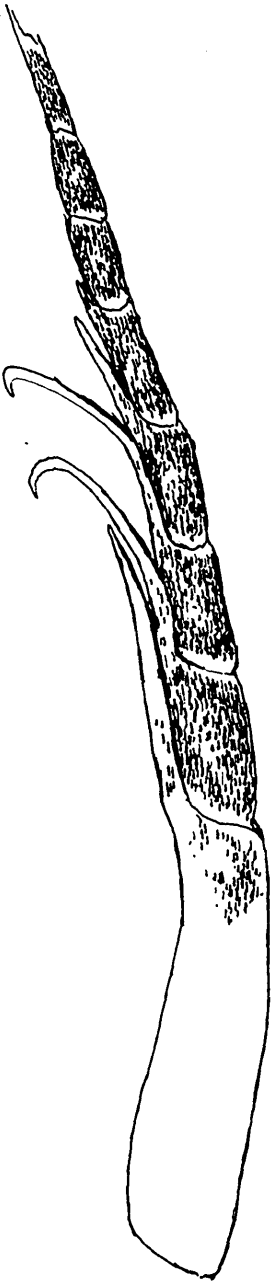
The green feathers of the rest of the underside and of the sides of the body, including the median under wing-coverts, have in general the same types of section-outline, also with some variation, but in adult birds the extreme triangular type is much rarer. Here also the pigmentation of the barbules is entirely yellow.

The feathers of the rump show in adult birds much broader outlines of the sections. Their ectal thirds are considerably broader than their ental portions (Fig. 13-15), but there is much less variation than in the ventral regions. The cortex is of more uniform thickness, and becomes very thin only in its extreme ental portions. Also in these feathers the basal portions of the barbs show a stronger lateral compression and a distinctly greater longitudinal diameter (Fig. 16).

As has been already mentioned by Steiner (1932), the barbules of the feathers of the rump show not only diffuse yellow lipochrome pigmentation but in their distal portions also a melanistic one. In the

material examined by me, this pigmentation generally occupies more than the terminal half of the barbule. Towards the base of the barb, the pigment becomes progressively limited to the tip of the barbule. As a rule, the pigmentation is so dense that hardly a trace remains of those nuclear spaces which in most birds appear between the melanin granules, as a lighter oval spot in the centre of each cell of the barbule. Towards the base of the barbule the granules disappear rather abruptly (Fig. 18). In the immediate neighbourhood of the cell-boundaries narrow spaces are left bare from the granules. These are often rather irregularly distributed, so that even in the terminal portions of the barbules densely pigmented cells may alternate with others having only a few scattered granules.

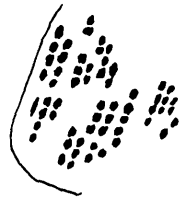
In most cases there occur slender, fusiform melanin rods, about $1,5\mu$ in length and $0,2\mu$ in width. Shorter ovoid granules in smaller numbers are scattered among these, about $0,9\mu$ in length and up to $0,6\mu$ in width. A few granules intermediate in shape and size between these two extremes are also present. In one male with very slight and irregular melanization of the barbules of the rump, the ovoid granules dominate. All granules are arranged with their longitudinal axis parallel to the axis of the barbule. The fusiform ones lie mostly close together so that they form more or less diagonal series where they are not so densely distributed (Fig. 19). The ovoid granules, on the other hand, often form bead-like series in parallel arrangement along the longitudinal axis of the barbule (Fig. 20).



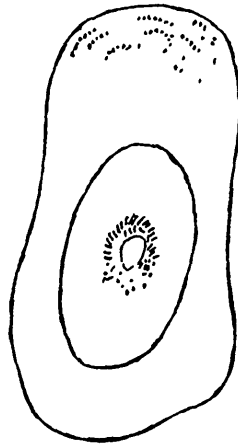
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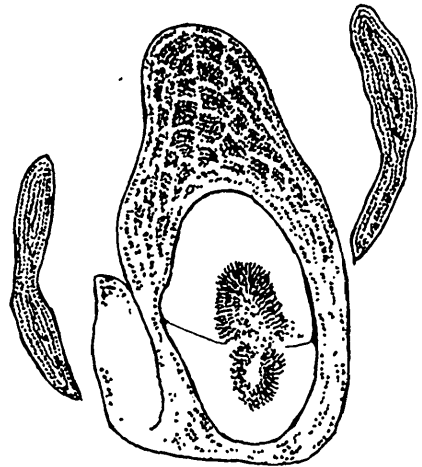
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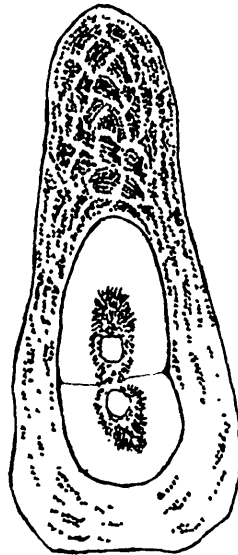
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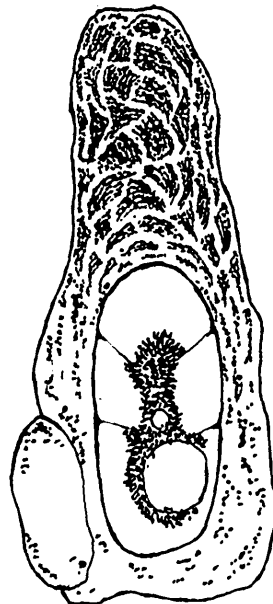
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- 18) Barbule taken from a rump-feather of the wild-type.
- 19, 20) Pigment granules of the barbules of rump-feathers of the wild-type,
- fusiform type (Fig. 19),
- bead-like type (Fig. 20).
- 21) Transverse section through a barb of a feather taken from the vertex of an adult wild-type; stage of transition between the most terminal yellow and the following black bar.
- 22) ditto., another feather of the same specimen: black bar following the most terminal yellow one.
- 23, 24) ditto., feather of another specimen: second black bar from the tip. In Fig. 24, a nuclear vacuole is cut.

(Fig. 18, 21 - 24: magnification $\times 1300$,
- 19, 20: - $\times 1500$.)

In young and in thyroxine-treated birds the shade of the green feathers on the underside is duller and on the rump distinctly greyish. Microscopical examination of the intact feather in reflected light shows that the barbs appear narrower than in the corresponding feathers of adult and normal specimens. The barbules appear more slender and of paler yellow colour.

Transverse sections of these barbs, even in the feathers of the rump, are triangular in shape and, particularly here, in the same section of a feather there may be present an irregular alternation of larger and smaller triangles. Generally, in agreement with the narrower appearance of the intact barb, smaller dimensions occur here. While the sections in Fig. 9, 10 show an average longitudinal diameter of 70μ and in their ental portions a transversal one of 35μ , in juvenile feathers the corresponding figures are 48μ and 22μ . The ectal crests are more distinct than in the triangular sections of adult birds. Even the basal sections of the barb show less the compressed parallelogram shape than that "of an inverted exclamation mark" with a rather sharp ectal crest. The barbules of the rump are also intensely melanized: in one thyroxine-treated specimen the granules are nearly all ovoid, arranged like beads on a string, some more than $0,6\mu$ in width. Sometimes a few scattered ovoids are to be seen in the feathers of the rump in various ental and lateral portions of the cortex.

The description of the outlines of the sections of the barbs and of the pigmentation of the barbules

leads to the question of their influence on the colour shade of the feather. If transverse sections of most types of intensely green, or intensely blue feathers are compared from all systematic groups of birds, there will generally be found rather broad oval, circular, or even transverse oval outlines. Bright green or blue feathers with a triangular or even a strongly compressed outline of the section of the barb are much rarer. Examination of the latter shows that either the barbules are not melanized (e.g., the pure yellow ones in the feathers of the underside described above), or that there are no barbules at all. In the former case, with broad oval outlines, intensely melanized barbules are the rule.

It is obvious that a broadened barb has an intensifying effect on bright structural colour, and counteracts the darkening effect of melanized barbules. This seems to be the case in the feathers on the rump of the adult wild-type of Budgerigar.

If the barbules are yellow, they do not disturb the bright colour. On the contrary, they seem to have some accessory function in fixing the bright shade. We see in fig. 9-11 that the lateral portions of the cortex are very thin, with the peripheral lipochroic zone scarcely apparent in many cases. If this type of feather is looked at from an acute angle, the lateral surfaces of the barbs are mainly seen. In this case we get an impression of a distinctly bluish colour undisturbed by the almost ineffective, thin yellow cortical zone in the lateral portions.

Even if this feather is looked at directly from above, still some of the lateral, not purely green

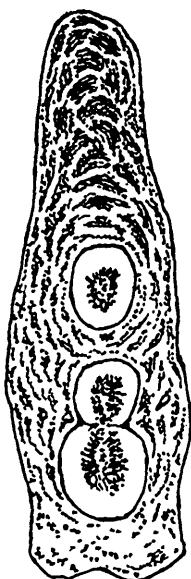
portions meet the glance, due to the triangular prismatic shape of the barbs. In this position, however, the whole ectal surface formed by the yellow barbules will also be seen, and the effect is to be compared with that of a piece of white paper closely ruled with regularly alternating blue and yellow lines. A glance made not too close at this object produces the impression of a distinctly green colour. Thus, a compound colour, may be produced or intensified not only in the typical way by the covering the one constituent colour by a transparent medium of the other one, but also by a close and alternating arrangement side by side of small surfaces of each of these constituent colours. Due to the fact that there is also yellow pigment in the cortex of the ^{barbs} ~~barbules~~, the feathers of the Budgerigar form only an approximation to this "side-by-side" arrangement, and no case is known to me in which an intensely green colour in feathers is produced entirely by this arrangement. (F)

F) At least in two cases shades of a bright lilac colour are due entirely to this "side-by-side" arrangement, viz. in two species of Parrots, the male of Psittacula cyanocephala (Linn.) and that of Geoffroyus personatus (Shaw). In both species the barbs of the occipital feathers show the same arrangement of the cloudy and the absorbing medium as in the Budgerigar, but the barbs are extremely compressed. The cortex of these barbs is colourless, and thus the effect would be a mere neutral blue colour, were it not that the bright red lipochrome pigment of the barbules provides the other constituent required for producing a compound violet shade.

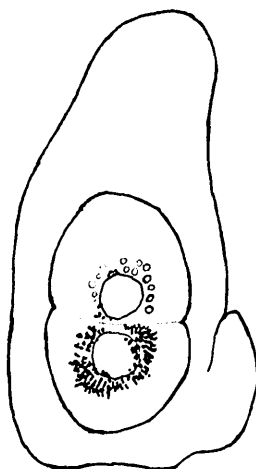
We have seen that in the juvenile Budgerigar the green colour of the underside is much paler than in the adult. In the young birds the barbs are much narrower, and, thus, the barbules form a much greater part of the visible surface of the feather. The barbules, however, are much paler yellow than in adult birds, and so the resultant is only a pale green colour. The greyish green shade of the juvenile feathers of the rump is, compared with the bright green of the adult bird, the effect of the narrow green barbs: these cannot counteract the darkening effect of the melanized barbules as do the broad ones in the adult. (F)

Apparently the ontogenetical change from the compressed-triangular to the broad-oval outline of the sections of the barbs in the Budgerigar might be the outcome of a phylogenetical one controlled by selection, as has been indicated in the footnote on pp. 18, 19. In the beginning of this chapter I have dealt with an adult specimen with feathers on the lower abdomen chiefly of the triangular type of section. The problem still remains, how far the appearance of this type in the adult stage is due to individual or racial variation, and whether there is any tendency to a gradual elimination of it even after the adult stage has been reached.

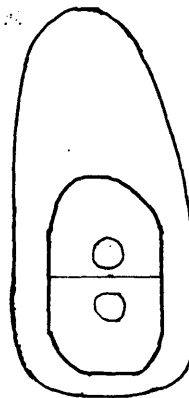
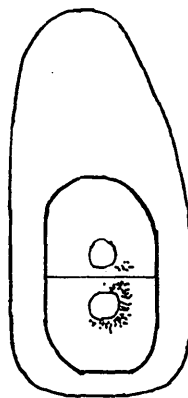
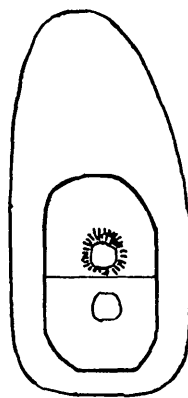
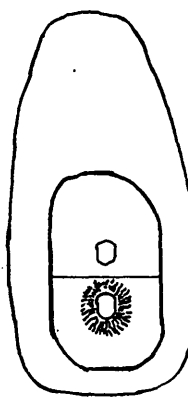
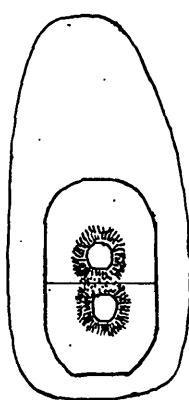
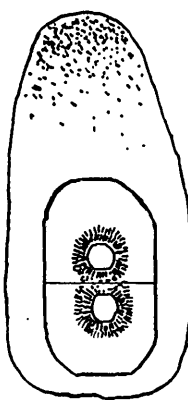
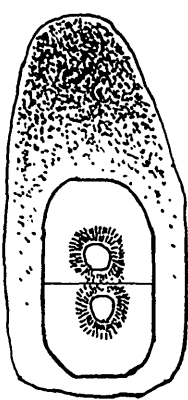
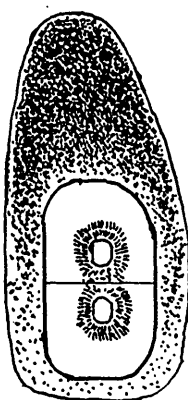
F) A dull olive green colour is produced often without the effect of any cloudy medium, merely by the "side-by-side" arrangement of compressed yellow barbs and intensely melanized barbules, e.g. in Parus major.



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- 25) Transverse section through a barb of a feather taken from the nape of the same specimen as in Fig. 22, section near the base of barb. The single medullary cells are separated by the cortical tissue.
- 26) *ditto.*, same specimen, same feather as in Fig. 22: stage of transition between the black bar, from which a section is represented in Fig. 22, and the yellow bar following in basal direction.
- 27) Diagram showing the transition between the black and the yellow bar of an undulated feather, in basal direction. The intermediate green stage is represented in the fourth drawing.

(Fig. 25, 26: magnification $\times 1300$.)

e) Undulated feathers, quills,
and tail-feathers.

On the structure of the undulated feathers, Steiner (1932) remarks that "the typical canalicular cells are much less well developed there and appear often to be displaced as a whole". An examination of a complete feather, e.g. of the vertex, shows, however, even to the naked eye, that almost regularly narrow ~~green bars~~ are intercalated between the black and the yellow ones: distinctly green lines are to be seen at the basal margins of the black bars.

Microscopical examination of the intact feather confirms this picture: we see that the green portions are due only to the barbs. At the boundaries between the green and yellow areas visible on the surface of the barb, an irregular but distinct pattern of small green spots on the yellow background is apparent.

The melanin granules in the cortex and barbules are generally not fusiform, ~~as are those~~ in the barbules of the rump. They are typical rods with rounded ends, arranged along the longitudinal axis of the barb or the barbule, and mostly in distinct longitudinal series (Fig. 36). Only around the spots occupied by the nuclei of the barbule cells before keratinization does the arrangement of the rods follow more or less the outline of the spot (Fig. 37). Owing to this arrangement, the rods appear only as round dots in transverse sections of barb and barbules (Fig. 21-25, 30, 32, 33).

In nearly all undulated feathers, at least in

the ectal parts, the medulla is composed of typical thick-walled cells with a round nuclear vacuole in the centre.

The feathers of the vertex, the cheeks, the hind-neck, etc. (Fig. 21-26) are rather small, and the barbs and barbules are proportionately small. In section the medulla shows a very small number of cells, arranged in a strictly uniserial fashion. In the portions of the barb which correspond to the black transverse bars, a continuous axial pigmentation nearly always overlaps more or less the cortical one (Fig. 21, 26), chiefly in basal but here and there also in terminal direction. The portions which have pigment granules in the axis but not in the cortex correspond with the narrow green bars mentioned above.

Finally, towards the pure yellow bars the axial pigment disappears gradually but in such an irregular fashion that incomplete pigment coronae occur in successive sections, partly in the ectal, partly in the ental portions (Fig. 26) of the axial part of the medulla. These sections correspond with the green-and-yellow mottled areas of the barb. In the pure yellow bars the transverse sections have no pigment granules at all, but possess the structural characters seen in the black and green bars. This structural uniformity explains the lack of any "damascene" pattern in albinos and lutinos of the Budgerigar. Such a pattern is bound to appear in all albinistic, melanistic, and other forms of species in which bars and spots are distinguished by structural as well as pigmentary differences.

The diagrammatic drawings of fig. 27 show the grad-

ual diminution of pigmentation from black to yellow bars in the axial part of the medulla as well as in the cortex. In the undulated feathers this cortical pigmentation shows a decrease from ental to ectal portions.

In the terminal portions of the barb of feathers of the regions enumerated above, the section is comparatively broad: the ectal portion of the cortex is considerably thickened but often not yet crest-shaped (Fig. 21). Also in these feathers the basal portions of the barb become more extended in ecto-ental direction, and more compressed (Fig. 23-25). Within this compressed medulla, the nuclear vacuoles are sometimes not proportionately small: then the pigmented axis appears deformed (Fig. 24). Here, however, I must emphasize that, in the undulated feathers of the wild-type, I have never found the medullary pigment anywhere reaching the inner wall of the cortex. Sections made very near the rhachis show the series of medullary cells sometimes interrupted by cortical septa (Fig. 25).

Before passing to the larger undulated feathers of the interscapular region and upper surface of the wing, a short description may be given of the feathers of the head which do not show undulation in the adult stage. The yellow feathers of the forehead show in transverse section an extremely compressed outline of the barb along its whole extent. The medullary cells are very small but of distinctly thick-walled type, with round vacuoles (Fig. 28). Comparatively long terminal portions of each barb do not contain medullary cells at all. At the extreme tip of the

barb, cortex and barbules contain a few melanin-rods, which produce on the feathers of this region delicate black terminal frills. As every feather-germ growing on the forehead of adult birds shows a black tip, we must assume that all the feathers of this region develop this delicate terminal melanization, and that its absence is due to the breaking away of the thin terminal portions of the barbs.

The yellow feathers of the cheeks and throat resemble those of the forehead. Their transverse sections, however, are comparatively broader, of more distinctly triangular shape, and with comparatively larger medullary cells.

The black cheek-patches are ~~due to~~ a single feather which shows this distinct mark in the centre of its surface. Sections of these feathers resemble those of the other feathers of the throat, occupying in their outline an intermediate position between those of the forehead and the undulated feathers of the vertex, etc. Within the area of the black patch, a distinct axial and a cortical melanization are to be found, like those in the transverse-barred feathers. In the cheek-patch, however, the axial pigmentation overlaps the cortical one only very slightly, and does not form any visible green margin around the black mark.

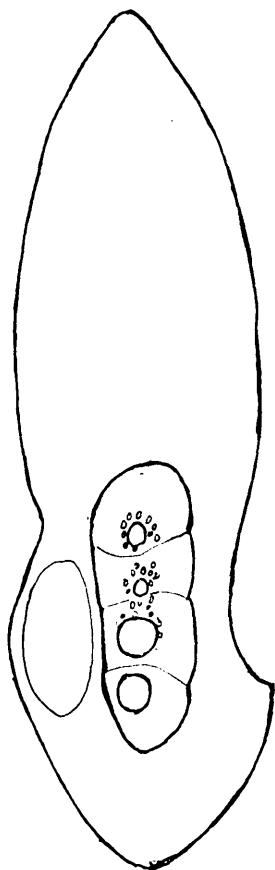
In the young bird, in which nearly the whole plumage of the head shows the undulated pattern, the feathers show the same structural and pigmentary peculiarities as in the adult bird. As in the green plumage dealt with in the former chapter, the juvenile barb is smaller in its sections, as well as generally more sharp-crested. The black cheek-patches develop

ontogenetically from the more undifferentiated undulated pattern in a peculiar way: while the surrounding feathers of the cheeks show from moult to moult narrower black transverse bars which decrease from the base to the tip till they reach the stage of practically uniform yellow colour, the feathers with the prospective round black patch retain the broad black subterminal bar. Gradually with each successive moult this mark develops into the round spot, mostly after the surrounding feathers have already become yellow.

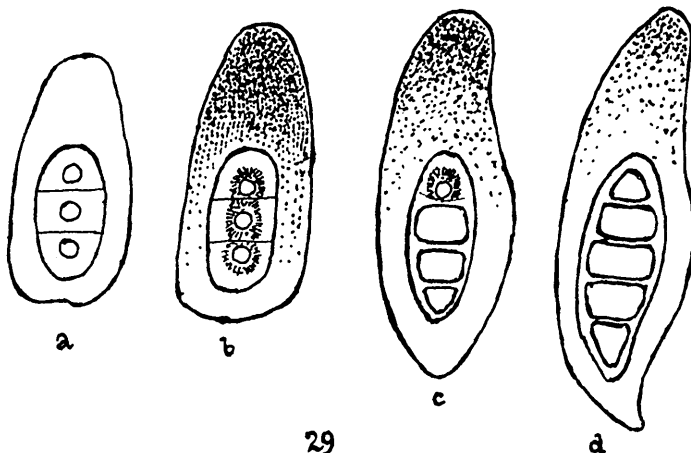
It is remarkable that in the female which I have already referred to as bred from introduced birds the melanin rods in the cheek-spots show a much more intense brownish colour than the granules in the undulated feathers. ~~Due~~ to the dense pigmentation, however, the shade of the spots appears as intensely black as in any other specimen of the wild-type.

The feathers of the interscapular region and the median upper wing-coverts show, besides their larger dimensions, generally no green transitory zones between the black and the yellow bars. Among the black bars only a broad subterminal one is very distinct, and in most cases only its terminal outlines are well-defined. ~~But specimens of the wild-type as well as of other~~ varieties occur in which the bar has distinct outlines on its basal side also. This seems to be an individual variation.

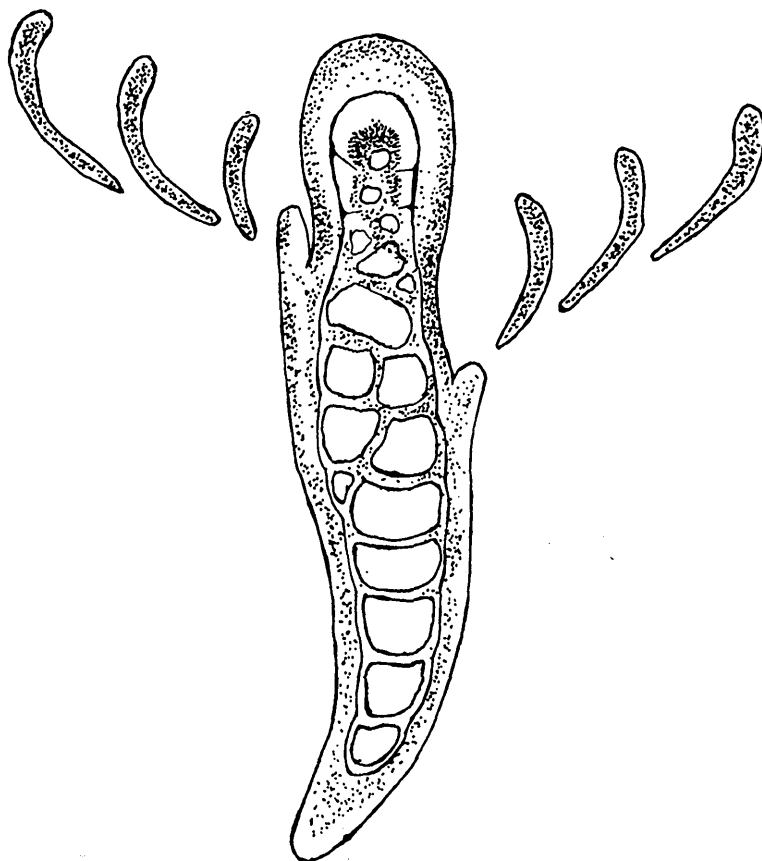
In these feathers the cortical pigmentation, which decreases from the ental to the ectal portions, mostly overlaps the medullary one towards the base of the barb in a more extended way than towards the tip



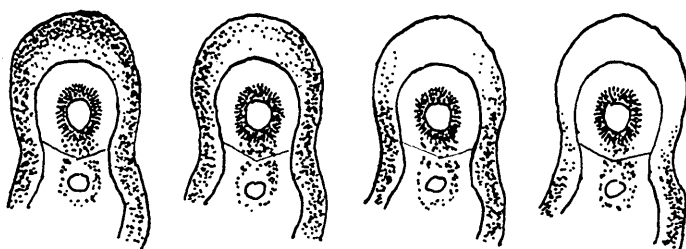
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- 28) Wild-type, same specimen as in Fig. 22: section through a barb of a yellow feather taken from the forehead, middle part of barb.
- 29) Semi-diagrammatic drawings representing the stages between the light terminal and the dark bar following it in basal direction, in a median upper wing-covert of the wild-type. In c and d, typical thin-walled cells replace the thick-walled ones in the ectal portions of the medulla.
- 30) Same specimen as in Fig. 22: section through a barb of the outer web of a distal quill, dark portion.
- 31) Diagram showing the transition between the black and the dark green portion in a barb of the outer web of a quill of the wild-type. (Only the ectal portions of the barb are represented.)

(Fig. 28: magnification $\times 1300$,
- 30: - $\times 800$.)

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(semi-diagrammatic Figure 29). In connection with the greater width of the barbs, the arrangement of the medullary cells is not always uniserial but rather irregular, at least in the medial and ental portions of the barb (Fig. 32). In the interscapulars, towards the base of the barb, the triangular outline, combined with lateral compression and uniserial arrangement of the cells, is also found (Fig. 33). In the broader sections the axial pigmentation is generally distinct only in its ectal portions, and slight and irregular in the ental ones. In the latter, the sections show here and there medullary cells which somewhat resemble the thin-walled ones, the morphology of which will be dealt with at the end of this chapter (Fig. 32).

Sections of the barbs of the median upper wing-coverts show, as in most other birds, some structures in common with the quills: even at some distance from their base, they show an ental ridge with the crest directed towards the rhachis (Fig. 29 c,d), although not so pronounced as in the feathers with high functional specialization (Fig. 30). In the barbs of the wing-coverts along with the presence of this ental ridge, thin-walled cells gradually replace the thick-walled ones in the ental portions of the medulla (Fig. 29): finally, near the base of the barb only thin-walled cells compose the medulla.

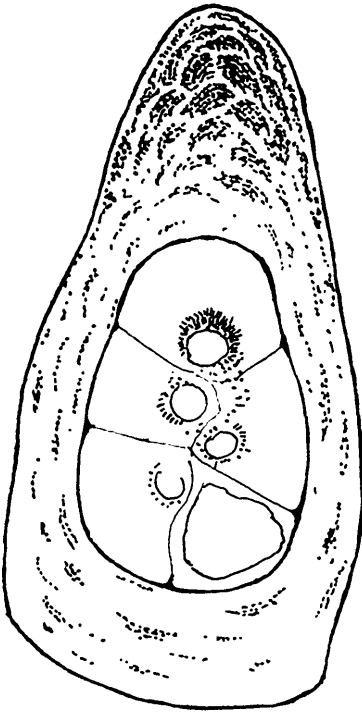
So also in the large upper and under tail-coverts, an ental ridge and thin-walled cells appear in the basal parts of the feather near the base of the barb. There the structural colour, which is green in the wild-type, is produced only by the ectal portions of the medulla, while the ental ones consist of apparent-

ly pigmentless thin-walled cells.

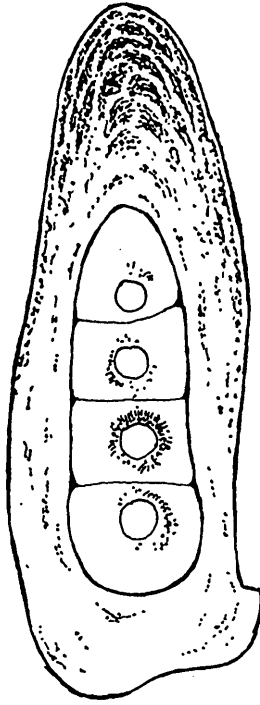
In the quills and rectrices of the Budgerigar, as in those of practically all birds, the ental ridge shows its strong functional development. From the level of the ledges of the barbules, the ental portion of the medulla consists only of thin-walled cells. In the Budgerigar the ectal portion is always composed of thick-walled cells which, as a rule, turn gradually into thin-walled ones in ental direction (Fig. 30). In some cases, however, in which the ectal portion of the barbs shows a strong lateral compression, its set of thick-walled cells is separated from the ental thin-walled ones by a cortical septum.

In these feathers, practically wherever the cortex is pigmented, a pigmented axis is present in the medulla. The latter, however, is distinct only in the extreme ectal cell. In ental direction, the axial pigmentation becomes gradually diluted. Sometimes, as in Fig. 30, a small number of pigment granules is still present in the thin walls of the ental medullary cells.

In those parts of the quills and tail-feathers in which on the ectal surface a dusky shade passes into dark blue or green, e.g. the outer webs of the distal primaries, the gradual disappearance of the cortical melanin granules takes place in a reverse way from that represented in the diagrammatic figures 27. and 29 : in the quills first the extreme ectal parts of the cortex become transparent, and this condition progresses in ental direction on both lateral surfaces of the barb, as may be seen in the diagram.



32



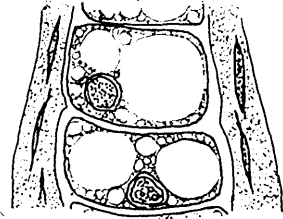
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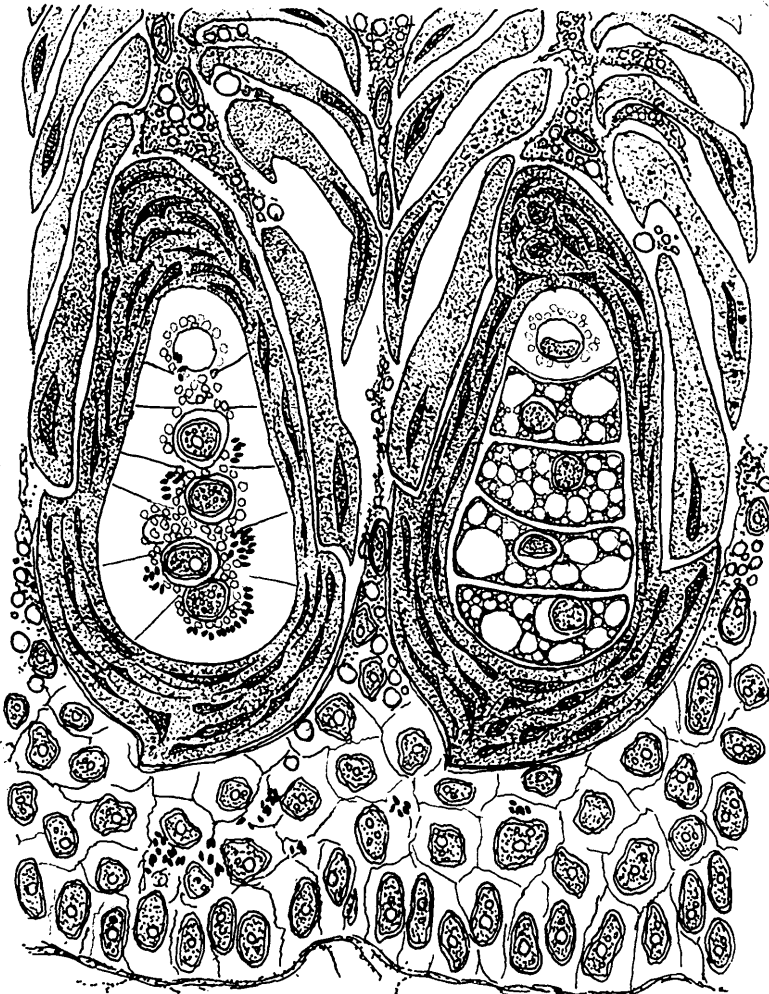
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- 32, 33) Wild-type, same specimen as in Fig. 22:
sections through barbs of an interscapular
feather.
- 34) Formation of cells of the medulla in a sec-
tion through the germ of an upper tail-
covert, taken from a partly leucistic bird.
In the figure, the right barb shows the
formation of four thin- and one thick-
walled cell.
- 35) Same specimen and feather-germ: formation
of two thin-walled cells with particularly
large vacuoles.
- 36) Rod-shaped melanin granules in a barbule:
of a quill.
- 37) *Idem.*, arrangement of the granules around
a former nucleus.

(Fig. 32 - 35: magnification $\times 1300$,
- 36, 37: - $\times 1500$.)

(Fig. 31).

The feathers dealt with above often show in the same transverse section both the colour-producing, canalicular cells, and the thin-walled ones which are much more common in all systematic groups of birds. Thus, we can compare the development of these two extreme types of medullary cells. As, in the wild-type and most other varieties, the pigment granules conceal a considerable number of structural peculiarities, we must again have recourse to feathers of albinistic or leucistic specimens.

Certainly in the Budgerigar there is no development of thin-walled cells, as Kniesche supposes, by extreme extension of a single vacuole in the centre of the cell, till the vacuole loses its spheric shape by fitting into the outline of the cell itself (p. 12).

Fig. 34 represents two sections of barbs, in the right one of which we see five medullary cells. Among these, the most ectal one is a prospective thick-walled cell, the remainder prospective thin-walled ones. In the former the nuclear vacuole is in the centre of the cell-body, the usual state in thick-walled cells when they are arranged in uniserial fashion. In close arrangement around the nuclear vacuole lie small "peri-nuclear" ones (p. 21), while the external portions of the cell-body show a granular but apparently solid structure.

In the four other cells, on the other hand, the nuclei are situated more or less eccentrically, while the whole cytoplasm contains a large number of very densely arranged vacuoles, some of them of much larger

size than the nuclear vacuole. The spaces between these large vacuoles are closely packed with smaller ones. In some cells, perhaps in those in a more advanced stage of obliteration, only a few very large vacuoles occur, occupying nearly the whole of the former cell-body, between a few strands of granular cytoplasm (Fig. 35). It is quite obvious that the eccentric situation of the nucleus in a cell, in these stages of specialization, is caused by the pressure of the growing vacuoles. In fact, more basal sections of the germ, representing the stage of development seen in fig. 7, show no vacuoles in the medullary cells, and the nuclei are in the centre of these cells.

Keratinization, after that of the cortex, begins along the external outlines of each of the prospective thin-walled cells. An apparently solid and distinct keratinous membrane arises which stains quite differently from the remainder of the cell-body containing the vacuoles. Conditions in the thin-walled cells are quite different from those in the thick-walled ones, between which, in the fully keratinized feathers, boundaries can easily be distinguished (Fig. 8). From the moment of keratinization the keratinous membranes seem to be so fused together that no cell-boundary is visible within them, although they arise from each of the bordering cells.

In many sections showing prospective thick- and thin-walled cells together, the former already possess an empty nuclear cavity within the incompletely keratinized cytoplasm (compare with p. 20). In the thin-walled cells, on the other hand, nuclei occur which still show almost normal structure and size, although

the cytoplasm is already displaced in great measure by vacuoles. It is, however, remarkable that in these nuclei nucleoli are absent or very small, while in those of the ental residual cells, as a rule, there is one large round nucleolus.

In the further stages of keratinization inside the medullary cell, contemporaneous with the obliteration of the nucleus, the network left between the vacuoles becomes more and more delicate. In some sections of completed feathers, traces of it are still to be seen. On the other hand, it is not easy to establish whether, finally, this network breaks down spontaneously, leaving only the cell-walls (Fig. 30), or whether the clear appearance of the interior of the thin-walled cells is an artefact due to the microtome-knife grinding these delicate structures without cutting them.

In germs and in completed feathers, however, some cells possess a more solid framework between the vacuoles. These are intermediate stages between thick- and thin-walled cells, the solid walls being rather thick, and the arrangement of the vacuoles an approximately perinuclear one. Further, within the series of the medullary cells in a section of a barb, instead of the usual gradual transition in ental direction from thick- to thin-walled type, there may be an irregular alternation between these two extreme types.

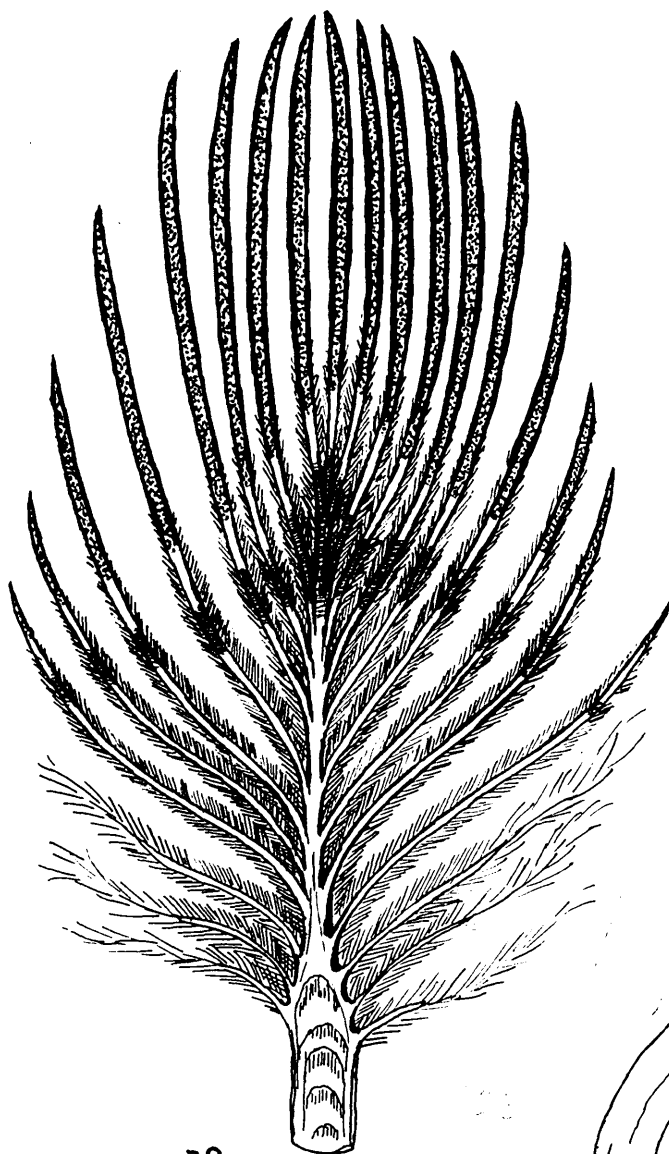
The medulla of the rhachis, even in the basal part of green feathers, is composed almost entirely of thin-walled cells of the extreme type. But, scattered among them is occasionally a single distinctly thick-walled cell with only one small circular vacuole

in its centre.

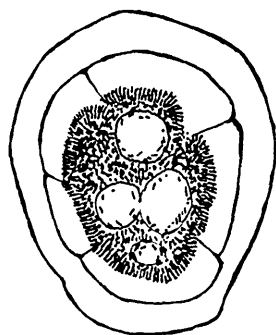
In no case did I observe the delicate pores seen by Kniesche in the walls of some thin-walled cells, and explained by him to be the extremely shortened radial channels (p. 12). Indeed, in the Budgerigar the walls of these cells seem to be of very solid structure.

Although thin-walled cells are the common type of medullary cell in rhachis and barbs throughout the class of birds, few particulars about them are given by authors. Schwann (1847), in his general histological work, gives a lucid account of the development of the feather of the Raven. He mentions the eccentric situation of the nuclei in the medullary cells of the rhachis, and, about the progress of keratinization, **makes** only the concise remark that "the contents of the cells at last dry up, and they become filled with air". About the nuclei of these cells, he adds that "they become smaller and smaller, and their outline more irregular, the nucleolus meanwhile remains". As mentioned on p. 22, **I can** confirm Schwann's statement about the nucleolus in the case of thick-walled cells. In the thin-walled ones, on the other hand, I have observed the reverse (p. 38).

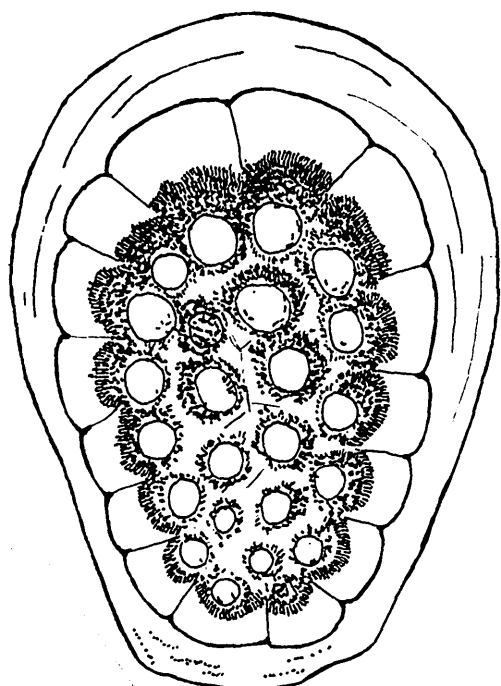
Davies (1888) says that in the medullary cells of the feathers of the Canary "the protoplasm accumulates apparently at the periphery of each cell which then turns into a hollow structure probably filled with fluid. Now the progress of keratinization begins in the plasma layer which encloses each cell". Both authors, however, emphasize that there is no cell-



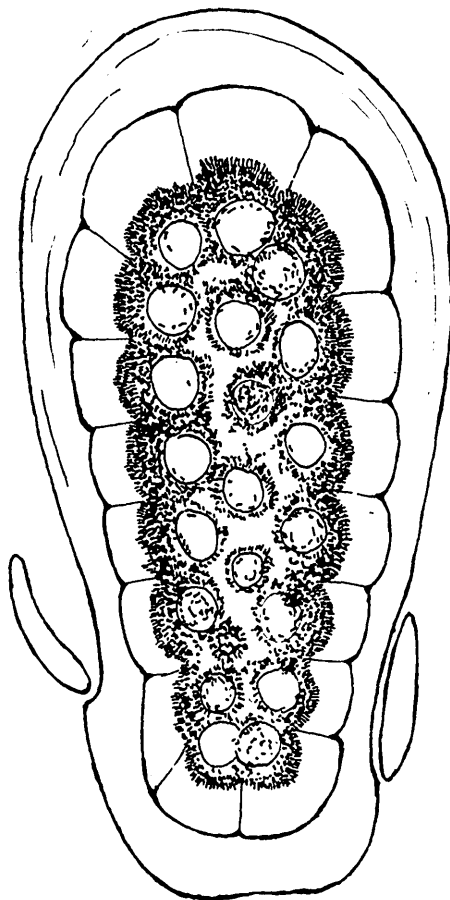
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- 38) Total view of a feather taken from the violet-blue cheek-patch of an adult wild-type.
- 39) Section through a rigid barb of a feather of the violet cheek-patch taken from an adult wild-type; terminal portion.
- 40) ditto., same feather, broadest portion of the barb.
- 41) ditto., same feather, nearer the base of barb, but still within the violet region.

(Fig. 38: magnification $\times 23$,
- 39 - 41: - $\times 1300$.)

boundary to be made out inside the keratinized walls.

None of the authors, however, gives further particulars about keratinization and the stages of emptying the interior of the cell, and even Strong (1902) refers only to Schwann. Nor do the instructive illustrations in Strong's paper give any insight into this matter. Kniesche, who has only dealt with full-grown feathers, apparently bases his hypothetical explanation of the formation of thin-walled cells (pp. 12, 13) on the above quotation from Davies.

The differences in the formation of these types of cells are apparently due to physico-chemical differences within the plasm. Greater speed in the chemical changes causing keratinization might produce the large quantity, irregular size, and irregular distribution of the gas-vacuoles in prospective thin-walled cells. On the other hand, a process more controlled by surface tension, because of its lower speed, might distribute the resulting gas more equally, by forming channels.

f) Feathers of the violet cheek-patch.

Although the bright violet-blue cheek-patch of the adult Budgerigar is very striking and its enamel-like appearance at once suggests some structural peculiarity, none of the authors have drawn attention to the feathers composing it. They show the bright colour and peculiar structure only on a comparative short terminal part (Fig. 38), and, except for the bright-coloured tip, they overlap. The concealed basal part is yellow, with one dark transverse bar, about

one millimeter distant from the violet tip. In the marginal portions of the cheek-patch, however, some feathers in transition to the neighbouring undulated cheek-portions have the violet tip shorter, and, correspondingly, the yellow basal part longer, with more than one dark transverse bar.

The general macroscopical appearance of the whole pattern-mark as well as of the single feathers recalls very much the rigid feathers seen in many Loriidae, particularly the violet feathers of the head of the Australian Trichoglossus swainsoni Jard. et Selb., and T. rubritorquis Vig. et Horsf.

Microscopical examination of the complete feather in reflected light shows that the violet terminal portions of the barbs are very broad, viz. 50μ on an average in their broadest portion. No barbules appear on the violet parts, although very short, dark pigmented barbules are visible where the violet colour passes through a short green zone into the yellow at the basis of the barb. The absence of barbules distinguishes this type of barb from the rigid one found in the Loriidae (Auber 1938). In the marginal feathers with more restricted tips, the violet terminal portions of the barbs are not as broad as those in the feathers within the mark.

The barred remainder of the feather shows, in its total aspect as well as in section, the same detailed structure as the undulated feathers of the head (pp. 29 ff.): as in those, the transitory zones between the dark and the yellow bars are slightly mottled with green.

Transverse sections of the violet portions (Fig.

39-41) show some interesting peculiarities compared with the green and undulated feathers. The cortical layer is colourless and transparent, except for a few melanin granules scattered in the ental portions of some sections. In the medullary area the subcortical cloudy ring has on the average the same width as that in the green feathers, viz. 7 to 9 μ in its ectal portions. The colour impression one gets when looking at the dry sections in reflected and in transmitted light (p. 9), are, however, rather different from those produced by sections of the green feathers. In the latter, the cloudy zone seems to be neutral or greenish blue in reflected and orange-red in transmitted light, while in the sections of the violet barbs the respective colours are a more or less violet-tinged ultramarine blue and a bright golden yellow. In comparison with the green feathers, therefore, there must be some differences in the canalicular structure, similar to those mentioned by Steiner (1932) for the "Dark" birds.

The axis contains a large number of nuclear vacuoles, corresponding with the rather large number of cells present in a section. The pigment corona of each vacuole in one of the peripheral medullary cells surrounds the vacuole more or less completely, but it is always considerably broader on the peripheral than on the central side. Owing to this fact, and since the peripheral nuclear vacuoles are very closely arranged in a circle, the pigment coronae of these vacuoles form a practically continuous ring beneath the peripheral cloudy zone. Towards the ental portions of the medulla, this cloudy zone and the dark absor-

bing one beneath it become gradually narrower, the gradual decrease of these two colour-producing media in ental direction being more obvious than in the green barbs where it is more irregular (Fig. 9-17).

The central part of the axis shows an almost uniform distribution of a large number of nuclear vacuoles, each of which occupies apparently the centre of the cell, and is surrounded by a pigment corona. Each corona is somewhat broader towards the periphery of the whole section, and in this part of the medulla the more ectal vacuoles show considerably broader coronae than the ental ones. We observe, therefore, the same rhythm in the distribution of pigment around the peripheral as well as the central vacuoles. Between the central vacuoles, the unpigmented portions of the cell-bodies give, in transmitted and in reflected light, the same colour impressions as the subcortical ring, when the dry section is examined. The portions surrounding the central pigment coronae form, of course, a more or less continuous network.

Examination of the barbs of corresponding feathers of albinistic and leucistic specimens shows that the medullary portions corresponding to the pigment coronae contain small circular "perinuclear" vacuoles. Since the light is totally reflected from the surfaces of these comparatively large enclosures of air, in absence of pigment the respective portions appear black in transmitted and milky white in reflected light, in dry sections.

The medullary pigment granules in the violet barbs are rod-shaped, the single rods about 1μ long, less than $\frac{1}{3}\mu$ broad. Here also the arrangement of the gran-

ules around the vacuole is generally radial, turning in longitudinal direction close to the surface of the vacuole. Although the shade of the pigment is very constant in the same feather, there is some variation between intense black, greyish, and more brownish shades. Moreover, in the adult female bred of an introduced pair previously mentioned, the granules in these barbs are distinctly light brown, of a much lighter shade than those of the circular cheek-spots (p. 33) which are also of a distinctly brownish colour.

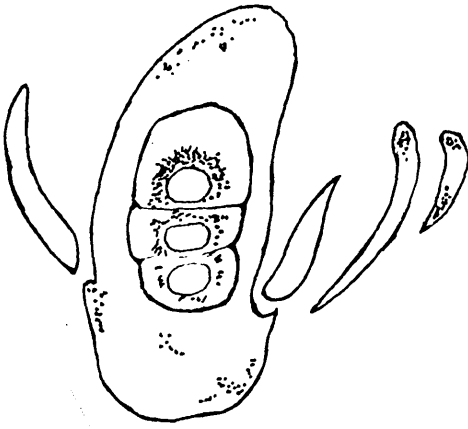
The pigment in the violet barbs of this specimen, however, cannot be a phaeomelanin, since it is practically insoluble in alkali: even a long boiling of the ground feathers in 40 % KOH has no effect on the dark granules which remain on the bottom of the test-tube after the whole keratinous structure has been destroyed.

Yet the structural shade of the cheek-patch of that specimen is hardly different from the colour in others, in spite of the difference in the colour of the dark medium. This fact is very striking, especially as Goernitz and Rensch (1924) attribute a number of cases of structural violet colour in bird-feathers to the effect of a cloudy medium beneath which are situated brown melanin granules instead of the typical black ones producing a neutral blue shade. This statement was made, however, before Steiner's description of the influence of differences in the canalicular structure itself (1932). It should therefore be useful to re-examine Goernitz and Rensch's conclusions from Steiner's point of view.

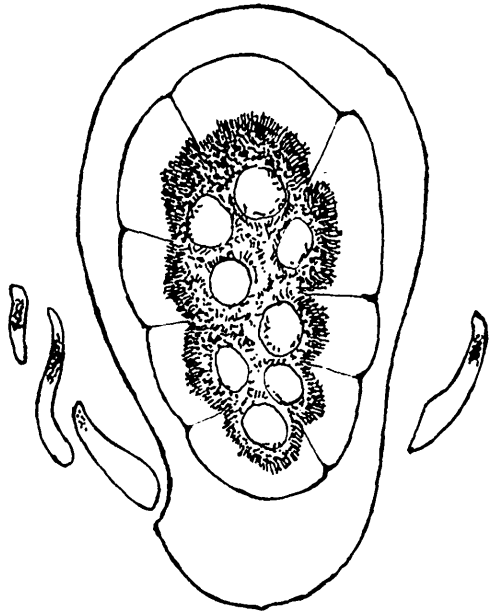
The outlines of the transverse sections of these barbs in the adult wild-type are very broad and rounded, the ectal portion of the section being always considerably broader than the ental. Near the tip of the barb the sections contain a small number of medullary cells and are nearly circular in outline (Fig. 39), whereas they are more oval in the broadest portions of the barb (Fig. 40). Where the latter narrows again, the sections become somewhat more compressed and their ental portions elongated (Fig. 41).

In the short green transitory zone between the violet terminal portion and the first yellow bar, yellow pigment begins to appear in the cortex. There the sections show a considerably smaller number of medullary cells with a more typical cloudy medium which gives the usual colour-impressions. The outlines resemble in size and shape those in Fig. 14-16. The sections of the basal, barred portions resemble in size, shape and structure those of the undulated feathers of the head.

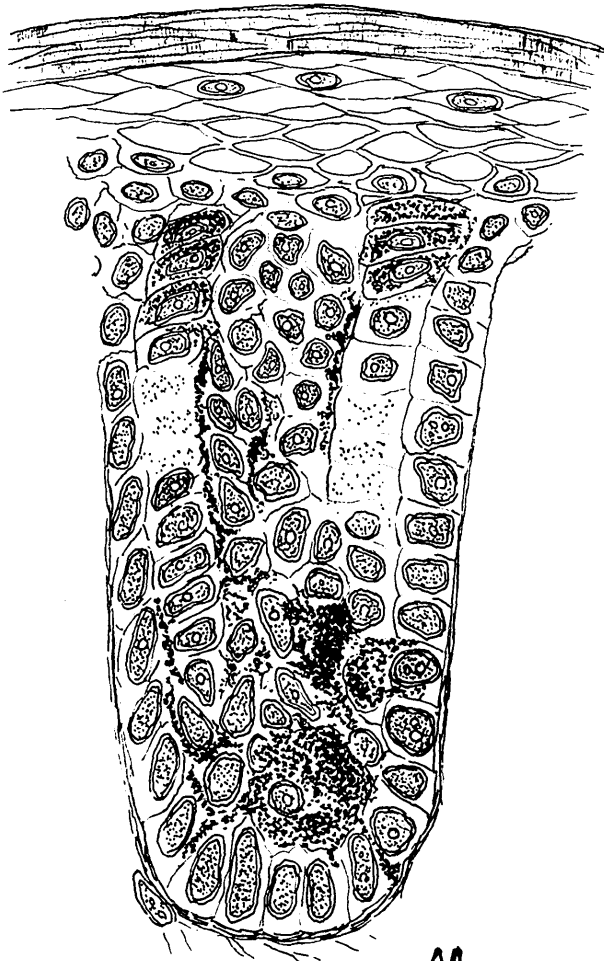
In the first plumage replacing the nestling down all undulations are very indistinct. In this stage the cheek-patch is either indicated only by a bluish tinge on the tips of the cheek-feathers, or by a more visible but still dull violet-blue mark, which, however, seems to be regionally more extended and not so definitely outlined as that of the adult bird. Apparently in the adult bird the mark is composed of a much smaller number of violet-tipped feathers than in the first plumage: in the course of the next moult, the marginal feathers of the mark are replaced



42



43



44

- 42) Section through a barb of the bluish terminal part of a cheek-feather taken from a wild-type in its first plumage.
- 43) *ditto.*, taken from a bird of the same clutch with a more distinct indication of the cheek-patch.
- 44) Early stage of development of a barb in a feather-germ of the cheek-patch taken from an adult wild-type: the nuclei in those cell-columns which preform the barbules appear partly indistinct.

(Fig. 42 - 44: magnification $\times 1300$.)

by more or less typically undulated ones without violet tips, and in the further moults a few of these are replaced by feathers with the round black spot, the remainder by pure yellow feathers (pp. 32, 33).

While the differentiation of the small number of feathers with the black spot requires two moult periods at least, the violet mark is practically completed after the first moult, as far as the examined material proves.

In the same clutch of about 8 to 10 weeks' old birds, one rather weak specimen, which was apparently a female, showed only faint bluish tips on the cheek-feathers. The other specimen, a few days older, stronger, and, according to the colour of its cere, apparently a male, showed more intensely violet-blue cheeks. In both cases, the corresponding barbs are considerably narrower than those of adult birds, and show barbules up to the tips. Fig. 42 shows a section of the bluish tip of a barb of the first-mentioned specimen: the outlines are compressed, the medullary cells small in number and arranged in uniserial fashion, and there are some pigment granules in the ectal portions of the cortex. Fig. 43, on the other hand, shows a section of a corresponding barb of the stronger specimen, taken at the broadest portion of the barb: it reveals a state more like that represented in fig. 40 and 41, but, in association with the smaller diameter, it contains a much smaller number of medullary cells.

After the moult, when the mark is already distinct and restricted, the barbs are still somewhat narrower and show more barbules towards the tips than those of

fully adult birds.

The modified differentiation in the germ of the violet terminal barbs without barbules would be of interest. But there is great difficulty in obtaining germs in such an early stage of development that their tip does not show early differentiation of the barb. Since the barbules are always formed before the barb, the stages showing the most atypical state during development would be those from the parallel arrangement of the cells inside the single ridges up to the first traces of the formation of the barb. Germs which have already grown beyond the level of the skin already show at their tip a clear differentiation of the ectal portions of cortex and medulla. (Fig. 45). As it has not been practicable to carry out excisions of skin with the germs still below its level, no exact results could be obtained.

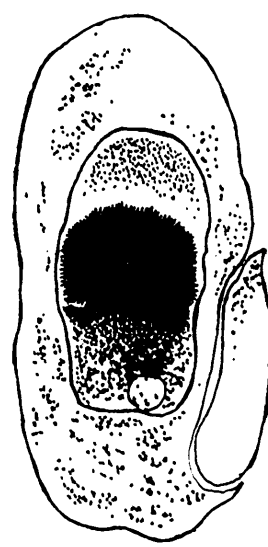
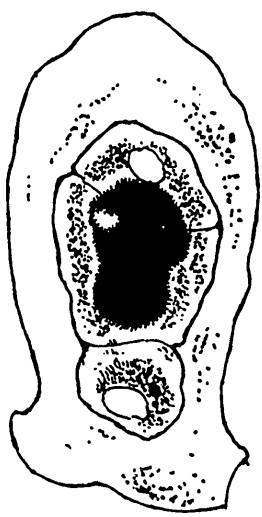
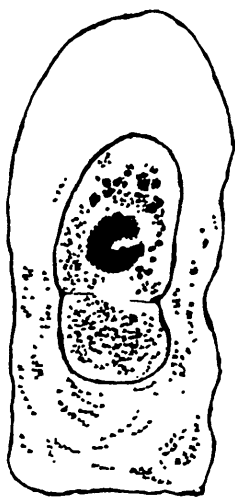
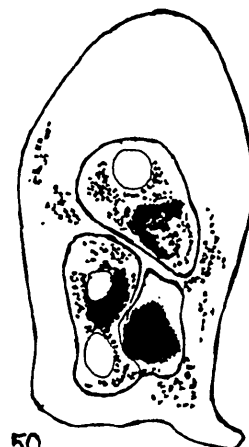
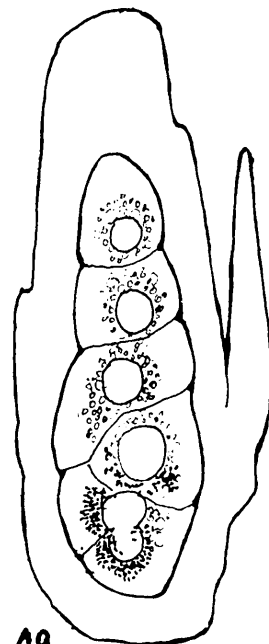
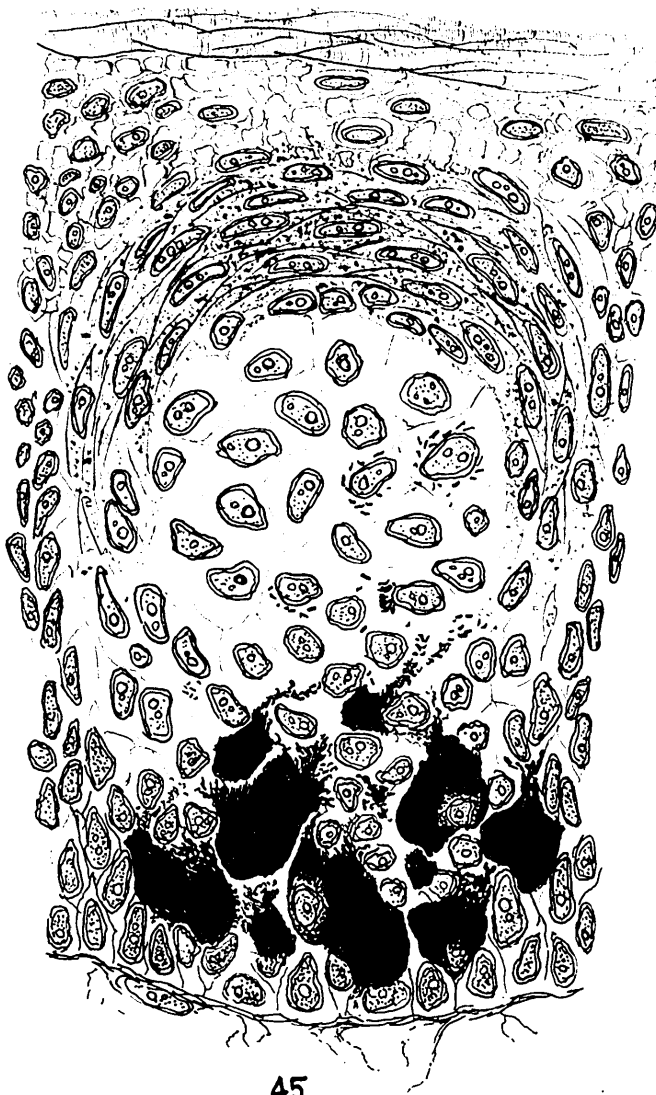
In one very short germ from this region, however, in which even the extreme tip shows no development beyond the stage in Fig. 45, an interesting detail occurs. Generally the double series of those cells which form the ~~future~~ barbules is very regular and their nuclei form uninterrupted rows in optical section, after having reached the stage of parallel arrangement. In this stage of the germ I mentioned, within the series of barbule-cells are irregularly scattered cells whose nuclei do not show the usual distinct outlines but seem to be only indicated by a slightly granular structure (Fig. 44).

Even in the areas without distinct nuclei, cell-boundaries can be made out, and, as the figure shows,

the most ectal cell-bodies of the series of barbule-cells already show melanization. The cells containing these indistinct nuclei may be considered to represent an early stage of degeneration taking place before the beginning of keratinization. Thus, the part of the germ seen in these sections may contain presumptive portions of violet barbs where the short and irregularly distributed barbules are inserted just before the bare terminal end.

Fig.45 shows a more advanced stage which corresponds to the bare tip of a violet barb. For the sake of showing the structure more clearly, a germ of a specimen with restricted melanization has been chosen. In the ectal portions of the presumptive barb we see the flattening of the nuclei in the cortical, and the extension of cytoplasm in the medullary region. In comparison with Fig. 6, it is apparent here that, due to the total absence of the barbules, the flattened cells of the extreme ectal zone of the cortex pass without interruption into the flattened cells forming the sheath: there is no isolating layer formed by polygonal ectal residual cells, as in typical barbs. In the same way, the presumptive barbs seem to be arranged very closely together, separated only by flattened cells. The triangular spaces between the ectal parts of the presumptive barbs are filled out with cells with rounded nuclei.

Particularly in the juvenile stage, the violet cheek-patch resembles somewhat the mark shown by many species of the Australian Psittacine genera Platyercus, Barnardius, and Psephotus. The feathers of the cheek-patch of these species, however, show no atypic-



- 45) Advanced developmental stage of a barb in a germ taken from the cheek-patch of an adult "cinnamon-yellow" bird.
- 46 - 50) Sections through barbs of a feather taken from the vertex of a "yellow" bird:
closely packed balls of pigment granules of irregular size and shape (Fig. 46),
halo-shaped dilute periaxial pigmentation (Fig. 47, 48),
basal section, showing only a few scattered granules in the axial portions of the ental medullary cells, resembling thus the corresponding sections of the wild-type, near the pure yellow bars (Fig. 49),
terminal section, showing the irregular shape and arrangement of the medullary cells, with cortical septa between them (Fig. 50).

(Fig. 45 - 50: magnification $\times 1300$.)

al structure.

g) The varieties "yellow", "whitesky",
and the "greywing" group.

Steiner (1932) interpretes these varieties as being phaenotypically characterized by a reduced quantity of pigment granules, compared with the wild-type. He explains the greenish suffusion of the feathers of the abdomen and the rump of the "yellow" bird by the presence of only a small number of melanin granules in the medulla of these feathers. This reduced absorbing medium would not be sufficient to produce an intensely green colour. About this variety, he further says that there is only a small number of isolated pigment granules within the cortex and barbules of the feathers of the back and quills.

Previously we have seen that in all undulated feathers and even in the dark portions of the quills both the cloudy and absorbing media are present: where the cortical melanin granules become scarce, a subjective colour impression of green is produced.

We may suppose that in a feather with the structural and pigmentary particulars represented in Fig. **22-25** the quantity of medullary granules was reduced proportionately and the cortical ones reduced to only a few scattered individuals: then, practically the state would be reached which is seen in the Figures **21** and **26**, and in the 5th to 7th **drawing** of the **diagrammatic Figure 27**, and the result would be a distinctly yellowish green colour, similar to that seen in the abdomen and the rump of the "yellow" bird.

In the body regions corresponding to the green zones of the wild-type, the complete feathers show an irregular pattern of distinct green and yellow spots, when examined in reflected light. This pattern is practically identical with that seen, under the same conditions, in the narrow green zones between the black and the yellow bars in the undulated feathers of the vertex, etc. (p. 29). Sections of the feathers of the abdomen and the rump of the "yellow" bird (Fig. 52) show a combination of the structure of the feathers of that region of the wild-type (Fig. 9-17) with an irregular distribution of reduced numbers of pigment granules changing from section to section.

The darker bars of the undulated feathers do not show the yellowish-green colour which would have been expected according to Steiner's interpretation. Their actual shade is a distinctly greyish one, when seen without using magnification. Microscopically examined in reflected light, these bars show a colour pattern consisting of more or less distinct but irregular black, green, and yellow spots. As in the wild-type, the narrow areas between the dark and the yellow bars show only green and yellow speckles. The barbules contain, as indicated by Steiner, only a few pigment granules with quite irregular distribution.

Sections of the portions of the barbs corresponding to the dark bars (Fig. 46-50) show that the pigment granules of the cortex are very thinly and irregularly distributed, and that there is no trace of the rhythm established in the corresponding sections of the feathers of the wild-type (Fig. 27, 1st to 3rd diagram). In the medulla, the arrangement of the

melanin granules is quite different from the axial one which can be seen in all feathers of the wild-type, and in those of the abdomen and the rump of the "yellow" bird.

In many sections, well-defined and more or less rounded masses of pigment granules occur: generally they are so dense that in their opaque interior no granular structure at all can be made out. Here and there the outline of these dense masses appears delicately toothed, so that they are seen to consist of typical granules. In size, the masses may vary from a diameter of only a few μ , consisting of only a few granules (Fig. 46), to groups either filling the axial centre of the medulla (Fig. 47) or nearly reaching both lateral walls of the cortex (Fig. 48). The eccentric situation of the nuclear vacuoles in many medullary cells (Fig. 47, 50) may have some connection with these dense accumulations of pigment granules. Beside these masses, loose groups of granules occur which may surround the dense ones, like a halo (Fig. 47), or fill out the spaces left between them. These scanty groups of granules are arranged irregularly, and not in the radial fashion prevailing in the axial pigmentation: owing to this irregularity, many of the granules appear in the sections as round dots, like those described for the cortex (p. 29), although they are really of the usual rod-shape. Like the dense pigment masses, these loose groups often extend to the inner wall of the cortex.

Further, we see that in these sections the uniserial arrangement of the medullary cells is sometimes disturbed. Here also, as may be seen in a



terminal section of a barb (Fig. 50), cortical septa may separate the single medullary cells.

Apart from these sections with atypical pigmentation and structure, many have complete or incomplete axial pigmentation and serial arrangement of the medullary cells, and resemble in every way the state represented in the 4th to 7th drawing of the diagrammatic figure 27 (see also fig. 49). Obviously, the atypical sections correspond to the black-spotted areas on the complete barb, and those with axial pigmentation to the green and yellow spotted ones.

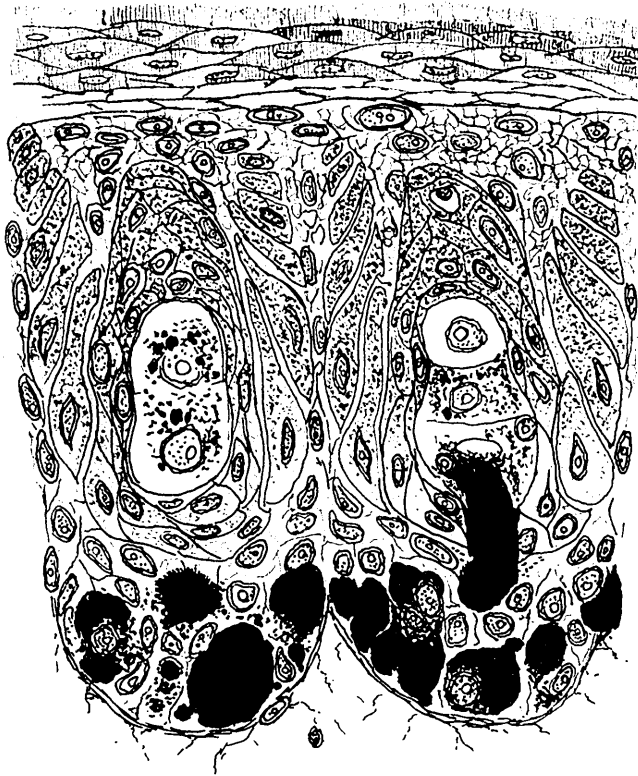
The terminal barbs of the feathers of the cheek-patch which is pale violet in these varieties show the same structure as in the corresponding barbs of the wild-type, combined with an irregular but distinctly axial distribution of the reduced quantity of pigment rods. This distribution corresponds in every way to that of the ovoids seen in fig. 52.

An explanation of how the state of reduced melanization in the feathers of the "yellow", etc. birds is brought about, has been given by Steiner (1932). Several years ago (1915), Lloyd Jones expressed the opinion that an autochthonous production of melanin might take place in the cells of the presumptive barbs and barbules themselves, in some examples of feathers of Pigeons in which typical melanophores cannot be distinguished between the ectodermal cells of the feather-germ. Quite independently of Lloyd Jones, and for other reasons, Steiner advocates an autochthonous formation of melanin inside the keratinizing cells.

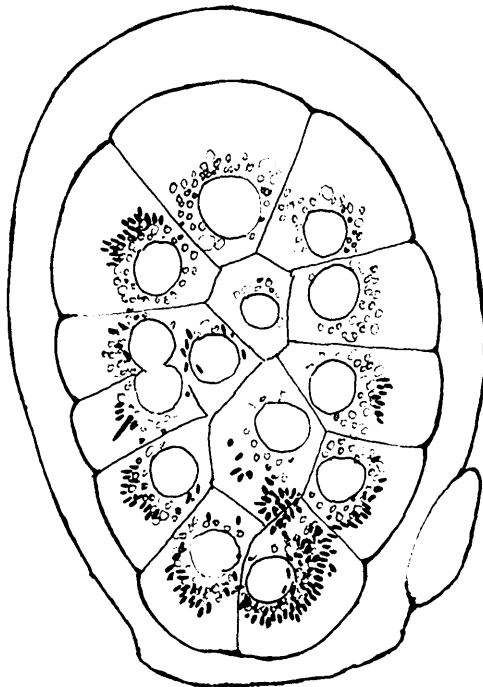
The classical conception, seen e.g. in Strong's

paper (1902) postulates a direct transport of melanin granules into the differentiated cells of the barbules, barbs, etc., by the distal portions of the pseudopodia of the melanophores. Steiner, on the other hand, draws attention to the difficulty of this conception, first, because of the impermeability for larger corpuscles, of the already keratinizing walls of the cells. Secondly, he shows that sometimes no direct contact takes place between the branches of the melanophores, on the one hand, and the pigmented keratinizing cells, on the other. As a first stage of melanization before the appearance of typical granules, he observes a transparent and diffused dark colour in both the presumptive melanophores and the cells of the barbules, etc.

Concerning the difference between the "normal" quantity of pigment granules in the full-grown feathers of the wild-type and the "reduced" one in the "yellow", etc. birds, Steiner shows that in the latter, between the presumptive ental residual cells, many rounded melanophores with short branches are distributed; in the "yellow" and "whitesky" specimens, according to Steiner's illustrations (his fig. 3 on p. 129), these masses of melanophores form a continuous, ring-shaped zone in the transverse sections of the germ, and the branches, in general, make no contact with the presumptive barbs or barbules. In the wild-type, on the other hand, the melanophores are smaller, discontinuously distributed in the epithelium, and extremely ramified, the branches being in a situation which seems to justify the classical "injection" hypothesis I have mentioned (see also



51



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- 51) Two barb-ridges in a feather-germ taken from the vertex of the same "cinnamon-yellow" specimen as in Fig. 45, in the stage after formation of the ectal portion of the barb. In the right barb, a broad branch of a melanophore is apparently entering the medulla.
- 52) Section through the terminal half of a barb of a rump-feather taken from the same adult "yellow" specimen as in Fig. 46-50.

(Fig. 51, 52: magnification $\times 1300$.)

Fig. 44). In the "greywing" group the condition is intermediate between the "yellow" and the wild-type.

Steiner, thus, concludes that in all these cases practically the same amount of melanin is produced by fermentation of tyrosine, within the whole epithelium of the germ: in the "yellow" bird an earlier and more intensive production of melanin in the melanophores would be, therefore, complementary to a more feeble one in the presumptive barbs and barbules which takes place later.

In the feathers of the abdomen and the rump, the quantity of the pigment granules is obviously reduced. On the other hand, in the undulated ones the dense masses I have described seem to be composed of a quantity of pigment which is greater than that present in the corresponding portions of these feathers of the wild-type, at least in the medulla.

The sections made of germs of undulated feathers of "yellow" birds, in the course of the present investigations, show some interesting details. Here, and also in other feather-germs of such birds, I have found many large and rounded melanophores in the ventral portions of the ridges, even between the cells of the basic cylindrical layer. In no case, however, do the melanophores form the continuous ring represented in Steiner's illustration.

In stages showing the medulla already outlined (Fig. 51), in germs of undulated feathers dense pigment masses are to be seen as I have described: there is a close resemblance between them and the smaller ones among the melanophores. In other sections (e.g. the

right one in the figure), a melanophore penetrates apparently into the medulla where parts of its lobopodium-shaped branch seem to be detached. In lower or higher sections, the same barb may show only an isolated spot of pigment corresponding in position to the distal end of the branch. It must be emphasized that in these ental portions of the barbs no sign of keratinization can be observed as yet.

Steiner's conception of the independent parallel pigmentation seems to be confirmed for the barbules and for the ectal portions of the barbs, by the present investigations, since often no contact of the pigmented portions of these differentiated parts of the germ with any melanophore could be traced in successive sections. The diffused dark coloration, however, mentioned by Steiner as being the first sign of pigmentation has in no case been observed.

However, the arrangement I have described in the medullary areae of the germ, namely the direct contact with the melanophore, the resemblance between it and the pigment masses as finally formed, and the state seen in the successive sections, make the classical conception of the development of pigmentation highly probable. At least, the dense masses peculiar to the undulated feathers of the varieties in question seem to originate in this way, while the more loose pigmentations around and between them possibly might be of autochthonous origin.

In all cases of axial pigmentation in the various varieties, developmental stages of the germ, in which the ental portions of the barb are not quite as differentiated as in fig. 7, show more or less continuous

strips of pigment granules between the axis and the ramified melanophores. In most of these cases, however, the resemblance of the pigmental arrangement in the medulla and melanophore is not so obvious as in the one described.

Certainly, sections of the completed feathers as well as of the germs show distinctly that the medullary pigmentation is an intracellular one. But the condition represented in Fig. 51 makes the penetration of the branch of the melanophore into the apparently not yet keratinized medullary cell nearly indubitable.

In the germs of other feathers of these varieties, e.g. of the violet cheek-patch (Fig. 45) the melanophores also show large, rounded bodies. Their branches, however, although short and hardly ramified, are narrower and acuminate. Thus, the medullary pigmentation of these feathers never appears in such dense masses as in the undulated ones.

The "greywing" birds show, in the medulla of the undulated feathers, the same conditions and apparently the same quantity of pigment as in the "yellow" and "white" ones. There is only a more intense but also irregular pigmentation in the cortex of these feathers in the "greywing".

The explanation hitherto given for the phenotypes of these varieties, namely that they are the effect of some factors reducing the quantity of pigment granules generally and proportionately throughout the whole plumage, must therefore be abandoned. On the contrary, here we must postulate factors which produce different effects in different regions of the

plumage.

h) "Dark" varieties.

The terms used for the varieties of this group, in the publications and notes given particularly by foreign breeders, are by no means fixed as yet, and in Steiner's fundamental paper no way out of this difficulty has been found. Causes of the confusion are not only differences in the description of the subjective colour-impressions made by breeders, but also considerable individual variation in the specimens themselves. Therefore, names of varieties used in the present paper are in strict accordance with those fixed by the Budgerigar Society.

The group comprises varieties in which the structural constituent of the colour is apparently shifted from the neutral blue region of the spectrum towards its violet end, and the intensity of the shade is decreased. ~~As in the case of the "Olive" variety~~ The haploid specific factors produce phaenotypes which are morphologically intermediate between the "normal" form (which in this case wholly lacks these factors) and the carrier of diploid factors.

Thus, a haploid introduction of these factors into the wild-type produces a "Dark green" bird, while the presence of two sets of these factors, under the same circumstances, produces the type known as "Olive". In absence of the yellow pigment in the cortex and barbs, the optical effects of these factors are much more obvious: then the "sky-blue" normal type turns first into "Cobalt", while the effect of diploid

factors is to produce the "Mauve" type (F).

Apart from these "chief" types, others occur in which obviously the factors "Dark" are combined with those of the group "yellow" etc., discussed before: thus we have in the lipochromatic series a "Yellow olive" and a "Greywing olive", farther a "Dark yellow" and a "Greywing dark green". In the alipochromatic series the corresponding varieties are the "White mauve" and "Greywing mauve", as well as the "White cobalt" and the "Greywing cobalt".

Duncker explained the characteristic shades in the "Dark" birds as resulting from the presence of phaeomelanin, instead of the typical eumelanin, in the absorbing medium. This explanation may have been influenced by the investigations made by Goernitz and Rensch (1924) mentioned above on p. 44.

Steiner, however, who made sections of full-grown feathers and feather-germs of "Dark" birds, denies emphatically the presence of phaeomelanin in the plumage of any variety of the Budgerigar. He ascribes the peculiar shades in the "Dark" feathers to structural changes in the cloudy medium itself. According to him, the feathers of the "Olive" and "Mauve" birds contain a much thinner layer of "cloudy cells" than occurs in the wild-type. In dry sections

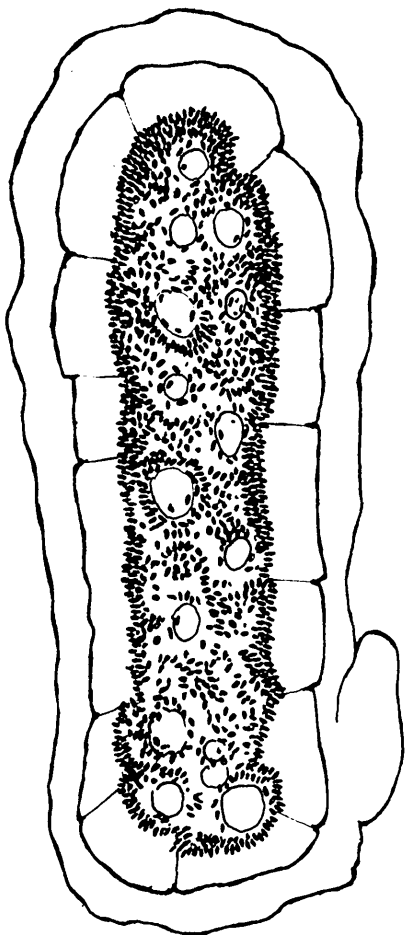
F) According to the names used by continental breeders, Steiner calls the alipochromatic varieties with the haploid factors also "violet", and even "mauve", while he uses for that with diploid ones mostly the term "grey". It is quite obvious that the birds described by Steiner are by no means identical with the "Australian violet", the "Dominant grey", and the "recessive grey", all these varieties showing quite different specialization.

this layer does not reflect the typical blue but a milky white light, and it transmits a pale brownish tint instead of the orange of the wild-type. Dark-ground examination of these sections carried out by this author proved that the canalicular structure in the "Dark" feathers is composed of more irregular and mostly much thinner channels than in the wild-type. This finding would be in full accordance with the physical one made by Lord Rayleigh (pp. 8, 9).

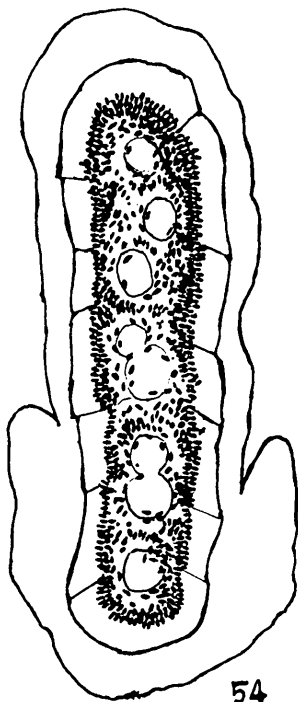
The investigations dealt with in the present paper led to a confirmation of Steiner's morphological results concerning the "Dark" varieties in essential, except for his histological interpretation of the layers, which diverges entirely from the findings made here (pp. 15, 16).

The axial pigmentation in the feathers of abdomen and rump is, indeed, composed of the same type of very regular and dark ovoids as is found in the corresponding feathers of the wild-type.

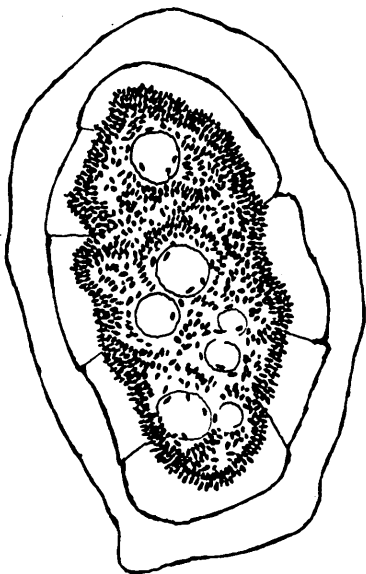
The colours of the cloudy medium seen in the dry sections, however, do not fully agree with the statement of Steiner mentioned above. In the "Olive" and the "Mauve" bird, these colours are the same as those described in the cheek-patch of the wild-type (p. 42), viz. a pale but distinct violet-blue shade in reflected and a golden-yellow one in transmitted light. The colour seen in reflected light is very similar to the shade of the corresponding regions of the plumage of the "Mauve" birds, which always show a distinct violet suffusion and are never neutral grey. In the "Dark green" and the "Cobalt" bird, Steiner describes the colour reflected from the sections rightly as



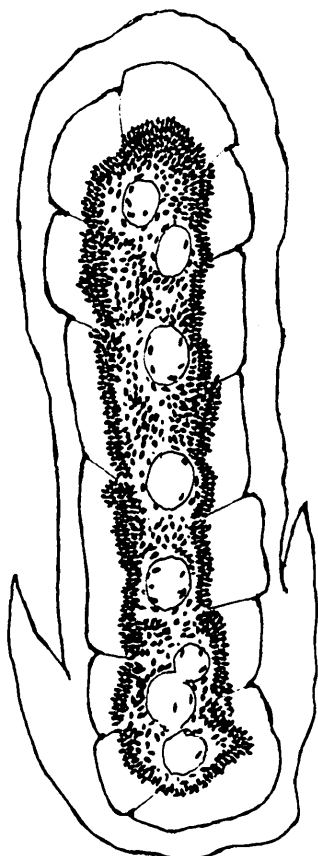
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- 53) Section through a barb of a rump-feather taken from a "Dark green" bird, near base.
- 54) ditto., rump-feather of an "Olive" bird, near base.
- 55) ditto., of another rump-feather of the same "Olive" bird, terminal section.
- 56) ditto., rump of a "Mauve" bird, middle part of barb.

(Fig. 53 - 56: magnification $\times 1300$.)

being "bright indigo- or cobalt-blue", while, instead of his impression of "brownish yellow-red" in transmitted light, an intense yellowish-orange colour has been met with which is distinctly different from the deep orange-red one in the wild-type. Possibly, the sections examined by Steiner were not quite dry, and therefore, showed duller and paler shades.

The outlines of the sections of the barbs of "Dark" feathers (Fig. 53-56) look, in fact, much more compressed and have much more shallow cloudy portions of the medullary cells, in comparison with the corresponding sections of barbs of the wild-type (Fig. 13-17). In the material examined from "Dark" birds, which consists entirely of fully adult specimens, no sections have been found to show an approximately triangular outline. In the same way, no periaxial vacuoles (pp. 16, 17, Fig. 8) could be made out.

As mentioned on p. 57, the difference between the wild-type, etc., and the "Dark" varieties, is not only a more violet but also a more dull shade of the structural constituent of the colour. The quality of the canalicular structure is, as already mentioned, the cause of the more violet shade. The duller element, on the other hand, is produced not only by the reduced width of the cloudy medium, as Steiner supposes, but also by the extremely compressed shape of the barb.

The effect of the thinner cloudy medium is the absorption of a greater quantity of light by the black medium situated beneath, i.e. seen subjectively, a darkening admixture of black with the bright structural colour in its own area. The narrower dimensions

of the barbs, on the other hand, restrict the bright-coloured portions of the visible surface of the whole feather, and render the indifferently coloured barbules as well as the shadowed spaces between the barbs more visible, as has been outlined on pp. 25-28.

It is characteristic of the "Dark" birds that, particularly on the rump, the feathers assume a somewhat brighter colour towards their extreme tips. Because of this, the region develops a somewhat regularly mottled pattern, a feature by which an "Olive" bird is very easily distinguished from a "green-Dominant grey" one (p. 101). This colour-effect in the "Dark" varieties is explained by the shape of the barbs, the terminal portions in which (Fig. 55) are considerably broader than the basal ones (Fig. 56). Thus the feathers expose a greater quantity of structural-coloured surface at their tips than at their bases, where, moreover, as far as the rump region is concerned, the dark-pigmented barbules are better developed. Since in the wild-type the basal narrowing of the barbs takes place more or less in the covered portions of the feathers (Fig. 13-16), the shade of the corresponding regions is more uniform.

The undulated feathers of the "Dark" birds show the same colour-effects in the cloudy medium as those of the abdomen and rump, in both reflected and transmitted light. The outlines of the sections of the barbs of the undulated feathers, however, are practically the same as those in the corresponding feathers of the wild-type: they show no more compression than is to be found in the wild-type.

In the "Yellow olive" birds and all other "Dark"

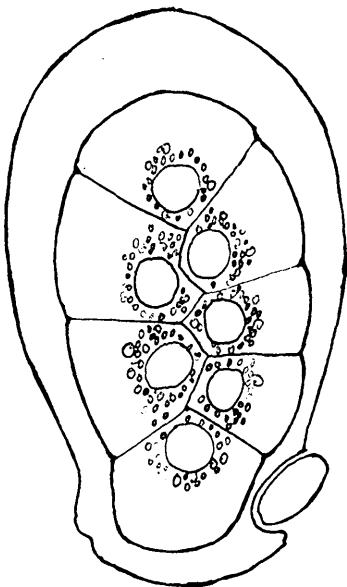
varieties, corresponding to the "yellow" and "grey-wing" ones, occurs the irregular pigment distribution peculiar to the latter in the body-feathers, as well as the dense pigment masses in the undulated ones.

We have seen that the cheek-patch of the wild-type has in its cloudy layer apparently the same type of canalicular structure as the body-feathers of the "Mauve" bird. That the former has a much brighter violet shade than the latter, is the obvious consequence of the greater thickness of the cloudy layer, the extreme width of the barbs, and the lack of barbules.

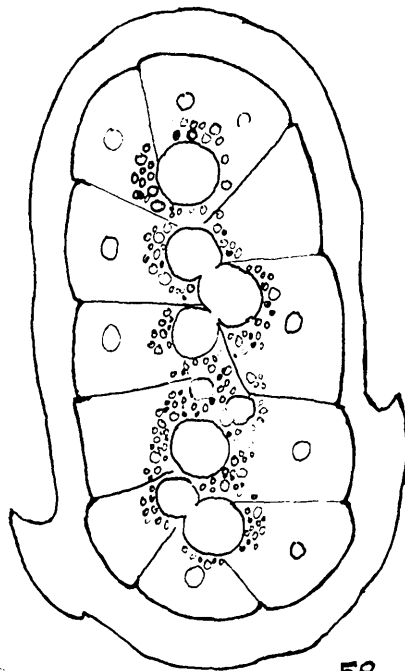
The cheek-patch of the "Dark", e.g. of the "Mauve" birds, shows a brighter violet colour than the body-feathers of this variety. Absolutely, it is less bright than the corresponding mark of the wild-type. The colour of this mark renders discrimination between an "Olive" and a "green-grey" bird rather easy, since the latter has a grey or bluish-grey, but never a violet-shaded, cheek-patch.

Microscopically, the barbs of this mark are narrower for about a third, but have the same shape of outline as those in the wild-type (Fig. 39-41). The subcortical cloudy zone is, on an average, slightly narrower than in the wild-type (6 to 7, instead of 7 to 9 μ), but here and there single cells show a reduced width of the cloudy medium up to 2,5 μ : in this way the outline of the pigmented axis has a somewhat irregularly scalloped appearance, quite different from that seen in the wild-type.

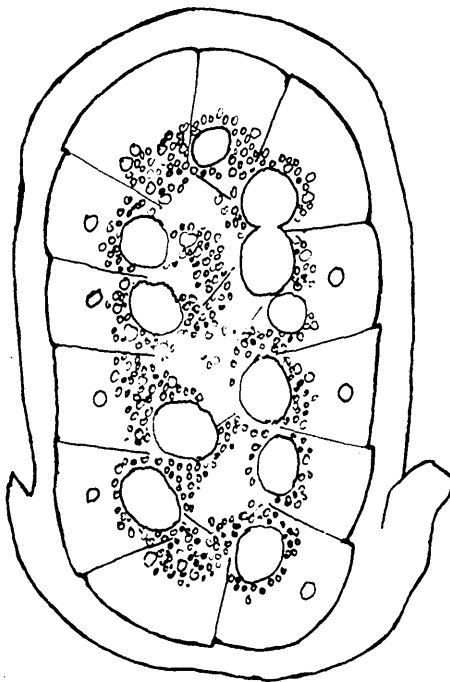
Summarizing the facts discussed above, we see in the "Dark" varieties a general replacement of the



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- 57) Sex-linked albino, section through a barb of a rump-feather, terminal half.
- 58) Lutino, lower abdomen, middle part of barb.
- 59) ditto., same region, another feather.

(Fig. 57 - 59: magnification x 1300.)

typical canalicular structure by a modified one and a wide-spread restriction of the dimensions of the cloudy medium, as far as the latter is optically effective. Thus we may conclude that the factors which are responsible for these changes have a general effect on the plumage of these varieties.

i) Albinos, lutinos, and partially
leucistic specimens.

Albinos, of which only sex-linked specimens have been examined, as well as the lutinos, do not show the least indication of bars or undulations, owing to the structural uniformity of the whole length of the barbs, even in those feathers which are undulated in other varieties, as already mentioned on p. 30. Specimens of both varieties show on the rump a slight bluish, or in the lutinos a slight greenish suffusion, when viewed at a small angle and particularly when the feathers are ruffled.

In section, except for the complete lack of melanin granules, the structural characters, and the colour-effects of the cloudy medium in reflected and in transmitted light, are the same as those described in the wild-type (Fig. 57-59). Because of the lack of the pigment granules, the cell-boundaries as well as the perinuclear vacuoles can be easily distinguished.

The feathers of the cheek-patch in the albinos contrast with the surrounding ones by their enamel-like appearance. In the lutinos they appear white with a very faint violet shade. Perhaps this is not entirely due to the effect of complementary colours:

since in both albinos and ~~lutinos~~ ^{lutinos} these feathers show the same type of canalicular structure as the corresponding feathers of the wild-type, the dark-shadowed spaces beneath the feathers might act slightly like an absorbing medium, and so bring about the violet hue. In the same way, the **larger** feathers of the rump, whose medulla has a greater diameter, might show the bluish or greenish suffusion seen there in certain conditions of light (F).

As one would expect, the feather-germs of the albinos and lutinos do not contain any melanophores.

A feather-germ of the upper tail-coverts of a partially leucistic bird was examined. The plumage of this specimen is irregularly and asymmetrically spotted, partly with pure white and partly with pale sky-blue, some feathers showing an irregular mottling of both of these shades. In the germ, within the presumptive ental residual cells, melanophores occur **only** in ental direction from such sections of barbs as contain some melanin granules within their medulla: the regions of the residual cells which correspond with pure white barbs contain no melanophores.

F) Kniesche (1914) mentions a similar case in which, in a bird-feather, a very slight bluish tinge is produced by a very well-developed and extensive **cloudy** medium only, without the presence of a proper absorbing layer.

j) Some remarks on the melanophores
in the dermis of the cere and, in
the pulp of the feather-germs.

Some authors discuss the origin of the melanin in the feather-germ. While Lloyd Jones has stated the probability of autóchthonous production of this pigment within presumptive portions of the feather itself and Steiner's investigations have made such a production probable for the cells of the barbules (pp. 52, ff.), a problem which has been dealt with already is whether the pigment originates in the epidermal epithelium itself, or whether it enters there from the dermal connective tissue.

Although Kerbert (1876) described in the skin of the legs of a Fowl embryo an obvious penetration of ramified melanophores from the dermis into the epidermis, no author has proved a similar progress in the germs either of down or of definite feathers.

Köl liker (1887) has established the presence of melanophores in the corium of many integumental structures. Therefore he concludes that the feather-germs also must ultimately receive their pigment from there. He describes, however, no actual instance in which he has found pigment within the pulpar portion of a feather-germ.

Rabl (1894) and Kawamura (1933) investigated the formation of pigment in germs of embryonal down, while Strong (1902) and Lloyd Jones (1915) did the same in germs of definite feathers of various species of birds. All these authors have not only established the formation of pigment entirely in epidermal cells, but they

also emphasize that in no case have melanin granules been found in the dermal pulp of a germ.

On the other hand, the presence of melanophores in the subepidermic tissue of the integument of birds itself has been established by some authors, although in none of these cases has a transmission of pigment from the connective tissue into the epidermis been mentioned (F). In all these cases, of course, the situation of the melanophores is apparently strictly confined to certain levels of the corium and they do not reach the basal membrane of the Malpighian layer. None of these cases, however, led to investigation as to whether pigment was present in the pulp of the feather-germs of the species in question.

Now, one would expect that the bright blue colour of the cere of the male Budgerigar would be produced by a structural arrangement similar to that described by Hassko for the Cassowary (see the footnote on this page). An examination made in the course of the

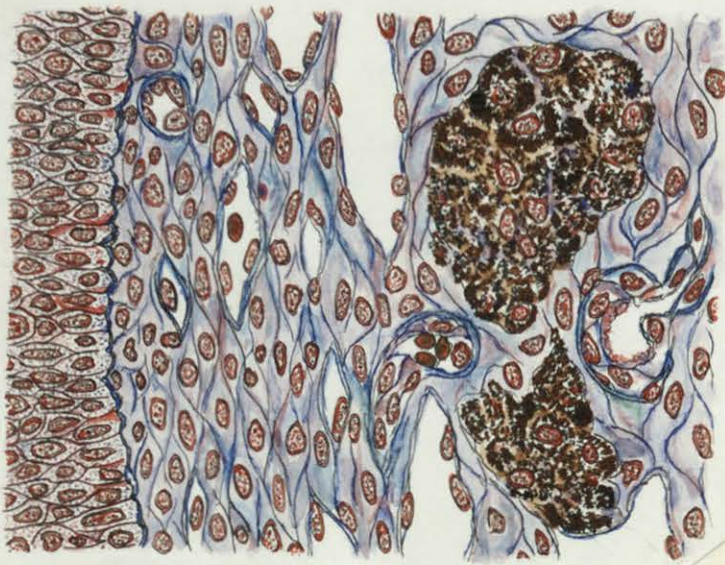
F) According to Hassko (1929) the bright blue colour of the skin of the neck of Casuarus is caused by the presence of a dense network of ramified melanophores, practically only in the superficial layers of the dermis. Lange (1931) has found delicately ramified pigment cells arranged around the capillaries within the superficial vascular layer of the dermis of the ventral skin of Corvus frugilegus, and I have found the same type of pigmentation in the skin of the chin of a specimen of Eos bornea guenbyensis (Scop.).

present investigations confirmed this expectation, to the extent that the colour is produced by the collaboration of a cloudy and an absorbing medium, and that both these media are situated subepidermically.

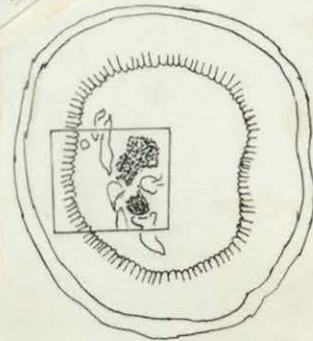
The situation of the absorbing melanophores in the cere of the Budgerigar, however, is comparatively less superficial than that described by Hassko for the much thicker skin of the Cassowary. In our material nearly everywhere beneath the Malpighian cells, the dermis develops a compact zone of comparatively great thickness and great uniformity of structure and width. In the sections examined, this zone is well outlined and here and there free from the contiguous zone. The latter might, for this reason and because of its very loose structure and thinness, be considered subdermic. Capillaries occur only within this layer where they are very closely packed. This subdermic layer passes immediately into the perichondrium of the external nasal capsule.

Melanophores appear practically only within the subdermic zone, mostly in a single and discontinuous layer situated immediately above the capillaries. In the perpendicular sections, the melanophores look like short dark streaks arranged parallel with the surface of the skin. ~~Apart from this~~ bulk of pigment cells in the subdermic region, a few scattered ones are also to be found in the deepest portions of the compact zone of the dermis. It is remarkable that this subdermic layer of pigment cells is continued through the nostrils into a corresponding pigmentation of the mucous membrane inside the nasal cavity.

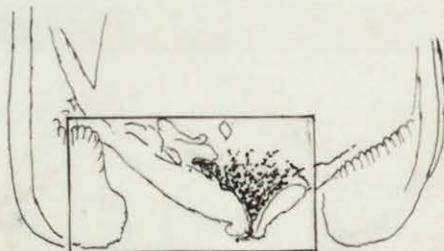
The existence of dermal pigment in this part of



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- 60) A portion of a transverse section near the base of a feather-germ taken from the cheek-patch of an immature "cinnamon-yellow" bird: melanophores are crowded near the centre of the dermal papilla.
- 61) Basal portion of a longitudinal section through another feather-germ of the same specimen, region, and stage as in Fig. 60: Strong's "closely packed dermal cells" are distinctly demarcated from the typical connective tissue forming the remainder of the pulp of the germ.

(Fig. 60, 61: magnification $\times 650$,
Fig. 60, - Fixation: Dubosq,
Staining: Azan.
- 61, - Fixation: Carnoy,
Staining: Azan.)

the integument led to the question whether pigment was present also in other parts of the dermis. Consequently the series of sections of feather-germs was examined from this point of view, and, in fact, here also melanophores were found sporadically.

The most striking example was in the extreme basal portion of some not very advanced germs of feathers of the violet cheek-patch of a cinnamon-yellow specimen. Transverse and longitudinal sections of these germs were made. Among the former (Fig. 60) melanophores occur in that part of the germ where the ridges are not fully developed, which does not yet contain pigment in the epidermal layer, and in which the cells of the connective tissue show the "closely packed" arrangement mentioned by Strong. These dermal melanophores are situated in the central portion of the pulp where they form a closely arranged and well defined mass. Each cell is round or oval, without the typical branches, and contains the same type of brownish-black granules found in the medulla of the barbs of the corresponding feathers. The arrangement of the granules within the cell is, however, not so dense as to render it impossible to distinguish the individuals. The nucleus is generally easily recognized: it lies near the centre of the cell and has the same round shape as the nuclei of the surrounding connective tissue.

Longitudinal sections made through the greatest diameter of the germ (Fig. 61) show further noteworthy particulars. In these sections it can be seen that Strong's "closely packed cells" occupy a rather restricted and distinct part of the pulp. This part

closes the umbilicus of the feather in the shape of a conic lid, apparently with a small opening on its top. In the peripheral parts of this cone, situated next the epidermal layer, the cells show an almost epithelial shape and large, oval nuclei. Within the umbilical aperture, the cells are extremely flattened and possess that densely fibrous structure which is found regularly in the compact zone of the dermis of birds. They contain nuclei of extremely elongated rectangular shape, apparently flattened by the pressure of the growing distal bulk of the connective tissue which is much looser: inside these nuclei as a rule no chromatin structure or nucleoli can be distinguished. The top of the loose connective tissue which fills the cone is formed by melanophores which also seem to stop up the small apical opening mentioned above. The conic lid itself seems to correspond with the compact zone of the dermis, while the loose remainder of the pulpar tissue might correspond with a spongy subepidermal zone which is not generally found in the integument of birds.

In a small number of other germs, e.g. of the rump and abdomen of different varieties, only single oval pigment cells were seen. They occur also in sections in which the formation of ridges is in its first stages. These melanophores show the single granules fairly well. The position of these pigment cells may be somewhat eccentric within the pulp but they never touch the basal membrane of the epidermal zone.

In a feather-germ from the cheeks of an adult

"recessive grey" bird (pp.103 ff.) another occurrence of pulpar melanophores has been noted, this time on the extreme terminal part of the germ. As indicated on pp. 31, 32, the feathers of this region show, in adult specimens of the wild-type and most varieties with "normal" melanization of the feathers, no melanin except for a very narrow black terminal frill. Throughout the tissue of these germs melanophores are produced only in the extreme terminal part which corresponds to that frill.

Transverse sections of this melanized terminal portion show that the pulp becomes gradually narrower while the epidermal tissue, consisting of the cylindrical cell-layer and the residual cells, becomes comparatively broader. The extreme terminal portions of the pulp form a somewhat flattened cord which is rather eccentrically situated inside the epidermal tissue and no longer well-defined from it. In these sections numerous rounded melanophores lie between the residual and cylindrical cells, and some are also to be found in the transition zone between the epidermal and dermal tissues, the boundary between which is not well defined. In the centre of the pulpar cord, however, no melanophores are to be seen.

In all these examples we have seen that the melanophores are rounded and do not show the ramification which would suggest a penetrative movement between the surrounding cells. In no case, except the last, in which further growth and cell-division are no longer probable, was a dermal melanophore seen in the immediate neighbourhood of the boundary between dermis and epidermis. Thus, there is no evidence of a penetration of melanophores from the connective

tissue into the epidermal epithelium of the germ,
and pigment production in each of these layers independently from the other is highly probable.

EXAMINATION OF SOME
NEW VARIETIES.

Since the publication of Steiner's paper (1932) some colour varieties of the Budgerigar have been observed which differ sometimes only slightly from those dealt with above, and which show, moreover, a great range of variation. The distinguishing features first observed in these specimens happened to be extreme instances, and thus were more easily made out. Because of their distinctness they were recognized by the Budgerigar Society as belonging to "new" varieties. However, it was shown subsequently that there occur all intergradations between these extreme cases and types already known. As a result, it happened sometimes that minor variations in the same direction came to be included under the "new" varieties, whereas they would have escaped observation, had the more extreme cases not attracted attention. Therefore it was thought desirable to include in the present investigations an examination of feathers of such extremely aberrant specimens.

On the other hand, some unmistakably distinct varieties have been produced which differ greatly from all others, in the tints replacing the typical green colour. Except for some cases of heterozygous specimens produced by cross-breeding, none could be considered a transition type leading to another variety. It is quite obvious that this second group of varieties required more intense examination than the first. Specialized features have been observed

in some of them which have no parallel in any bird-feather so far examined.

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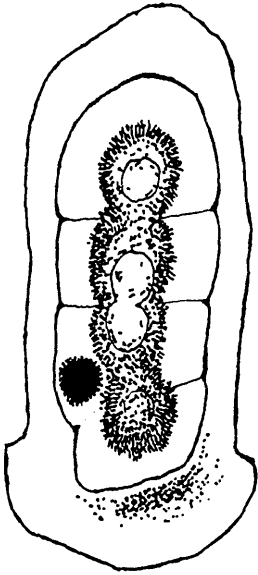
The "Bluewing",

The general appearance of this variety resembles that of the "whitesky" (pp. 49 ff.). The greyish undulations of the latter variety, however, are more or less replaced by skyblue ones. In most cases this replacement is obvious in the head and neck, while ~~on~~ the wings the shade of the bars generally gives the same greyish impression as in the ordinary "whitesky", in spite of the breeders' term "bluewing".

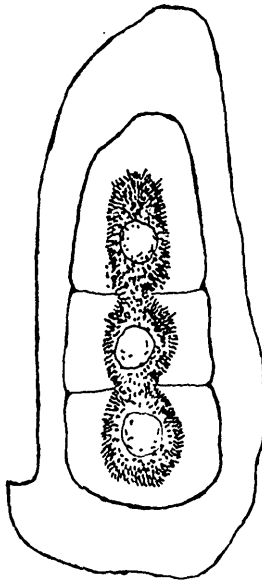
In one specimen considered a typical "bluewing", in general the head and neck show only blue undulations. There are some feathers in which this colour is also extended over the portions corresponding with the white bars in other feathers of the same region. In these and in the other feathers of this region, however, the hidden basal portions show here and there greyish bars.

Sections of a feather with extended blue colour show a thinner cortex than that observed in corresponding feathers of the wild-type and the "yellow" group, showing in this respect an approximation to the state found in the feathers of the abdomen. An extremely high ectal crest is never present even in basal sections (Fig. 63), and the sections seem to be slightly larger than those of the corresponding feathers of the ~~varieties mentioned~~.

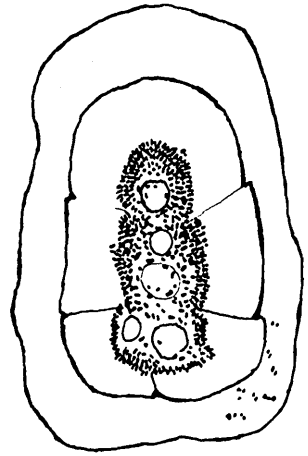
According to the extended and intense blue colour of this feather, a complete pigment axis can be seen in nearly every section. Only in the lighter apical portions some sections show an incomplete axial pigmentation, mostly without pigment in the ectal medullary cells. In the sections corresponding to the



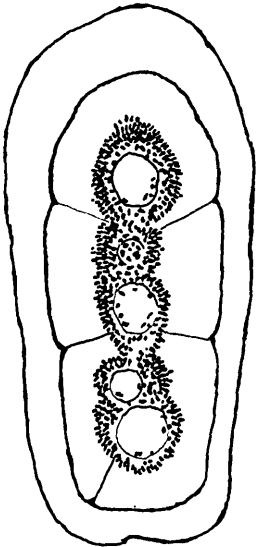
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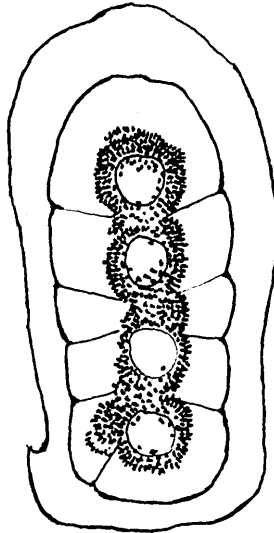
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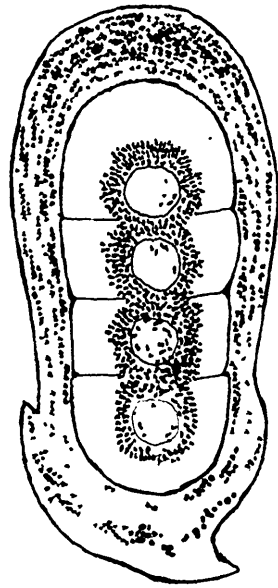
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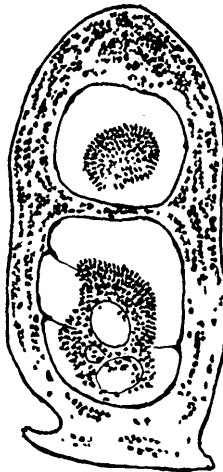
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66



67



68

- 62) Section through a barb of a feather taken from the vertex of a "bluewing" specimen, middle part of barb.
- 63) ditto., same feather, near base.
- 64) Section through a barb of a feather taken from the vertex of a "Cobalt opaline" specimen, middle part of barb.
- 65) ditto., same feather, near base.
- 66) Section through a barb of the upper wing-covert taken from the same "Cobalt opaline", broad blue terminal bar.
- 67) ditto., black subterminal bar.
- 68) as in Fig. 67, another barb near base.

(Fig. 62 - 68: magnification $\times 1300$.)

hidden greyish bar, some dense eccentrical pigment masses can be observed (Fig. 62). These masses, however, are by no means as frequent as in the "yellow" bird. In some sections, a cortical pigmentation is present. As in the "yellow" group, this pigmentation is found chiefly in the ental portions, but it is much more dense than that found in any corresponding feather of a "yellow" or a true "whitesky" bird.

Some specimens occur which might be denoted "greenwing", "cobaltwing", etc. A combination of the generally extended cortical pigmentation of the "greywing" group with the more regularly axial one of the "bluewing" would appear possible: macroscopically, however, such birds might not differ in their appearance from true "greywings", and, therefore, they might have escaped the attention of the breeders.

Thus, except perhaps for the thinner cortex, the differences between the undulated feathers of the "bluewing" group on the one hand, and the "yellow" one on the other, may be considered as due to only slight variations in the medullary pigmentation, ranging from irregular masses to regular axial arrangement of the granules.

The "Opaline".

Specimens belonging to this variety may show a very striking pattern. The undulation on the head and neck is almost absent: it is, in extreme cases, only represented by the narrow black terminal frill on each feather of this region. The latter shows, in specimens which correspond in their pigment qualities and canalicular structure to the wild-type, a more or less intense green suffusion on the yellow background. On the wing-coverts and the interscapulars the black markings are somewhat reduced: mostly only the black subterminal bar is found on each feather, and this mark is relatively very narrow, rendering the light terminal bar much broader. The latter and the basal part of the feather are almost as bright green as the abdomen, instead of yellow. On the basal half of each quill, the outer web, too, shows an extensive yellowish or greenish white mark, and the central rectrices may be whitish along the shaft. The round black spots on the cheek may vary considerably in size (F). In some specimens the downy basal portions of the feathers of the rump are pure white instead of grey. In the terminal half of these feathers, the barbules do not contain any melanin granules.

F) The efforts of the breeders aim at the rearing of specimens in which these marks are much larger than in the wild-type.

These features may occur combined with those of any other of the chief types dealt with so far. In every case the light undulations on the wings show the same colour as the abdomen of the respective specimen, and, in the same way, a suffusion of this shade may appear on the vertex, occiput, and hind-neck, while in other specimens these regions may appear pure yellow or white, according to the presence or absence of the yellow pigment.

Of a "Cobalt-opaline" specimen with a very intense suffusion of cobalt blue and only indications of black frills on the head and neck, a feather of the vertex has been examined. The transverse sections of the barbs show a relatively broader outline and a smaller vertical diameter than those of the wild-type (Compare Fig. 64, 65, with Fig. 21-26). Owing to these features, the more extended medulla, and the but faint indication of the ectal crest, the similarity to the outlines of sections of abdominal feathers is here much closer than in the corresponding feather of the "bluewing" (p. 74, Fig. 62, 63).

In the median upper wing-coverts of this "Cobalt-opaline" specimen the same features seem to be maintained, as shown by Fig. 66-68, compared with Fig.

29: as far as the examination has been carried out, only thick-walled cells have been established in this feather of the "opaline" bird.

In accordance with the bright and intense structural colour which replaces the white or the yellow in these feathers, the medullary pigment distribution in them is a strictly and completely axial one: none of the eccentric pigment masses typical of the

"yellow" group and occurring also in the "bluewing" have been found here. Except for the pigmentation on the terminal ends of the barbs, the feather of the head shows in the cortex only here and there a few granules in the ental portions. Within the extent of the black bar the examined wing-covert also shows a complete axial and a rather intense cortical pigmentation of a density somewhat decreasing towards the ental portions.

In contrast to the pigmentation of the corresponding feathers of the wild-type, which is composed chiefly of granules of the rod-type, in the feathers of the opaline ovoid ones seem to prevail. These ovoids, however, are much smaller than those found in the green feathers of the wild-type.

A feather of the rump of the same specimen shows, as has already been mentioned, no melanin in the barbules. The sections of the barbs of this feather are shorter but comparatively wider than those of the corresponding feathers in "Dark" varieties (p. 60). The cloudy zone in this feather has the usual width found in those of typical "Cobalt" birds. The arrangement of the medullary cells is, on the other hand, not the prevailingly uniserial one seen in Fig. 53-56; it shows, of course, some approximation to the state shown in Fig. 52.

The feathers of the cheek-patch do not differ from those of other "Cobalt" birds, the thickness of the bright-coloured barbs being smaller than that of the wild-type (p. 62). There are, however, no black markings in the basal, typical portion of this feather of the "opaline". Their absence is correlated with

the lack of undulation in the feathers of the head and neck in this specimen.

Sections of feathers of the white forehead show the same outlines as the corresponding ones in the wild-type (Fig. 28).

As the examination of this variety was carried out on **limited** material, the results will require further confirmation. There is, however, a certain tendency to reduce the development of the cortex and the melanization of this layer and of the barbules on the one hand, and to increase that of the medulla and of the axial pigmentation, on the other. There is, however, no proof of a general influence of this tendency on the plumage (F).

F) Steiner (1939) recently describes a "lively opalescent gloss throughout the whole plumage" in opaline birds. The specimens I have seen do not show any such feature. Steiner, however, **may** have examined birds provided by continental breeders, while in Great Britain this variety originated independently. The feature mentioned by him might be caused perhaps by a more intense and general decrease of the thickness of the cortex and increase of that of the medulla.

The "Clearwing" Group.

Specimens of this group show in their external appearance a combination of the intensely coloured underside and rump typical of the wild-type, with the greyish pattern of the undulated regions, the quills and rectrices, seen in the "greywings".

In other cases, underside and rump show the somewhat lighter shade seen in these regions in the "greywings", while the undulated regions, etc., are as very pale as in the "yellow" or "white" birds.

Finally, we may see combinations of an intensely coloured underside and rump of the wild-type, etc., with an extremely pale undulation of the "yellow" type.

Such regional combinations may occur in birds with or without yellow lipochrome as well as with typical or "Dark" structure of the cloudy medium throughout the whole plumage.

When examined microscopically, the undulated feathers of "clearwing" specimens resemble closely the corresponding ones of "greywing" or "yellow" birds (Fig. 46-50). The feathers of the underside and rump, on the other hand, resemble those of the corresponding regions in "wild-type" or "greywing" specimens.

We must, therefore, consider the factors which produce these changes as having a strictly regional effect.

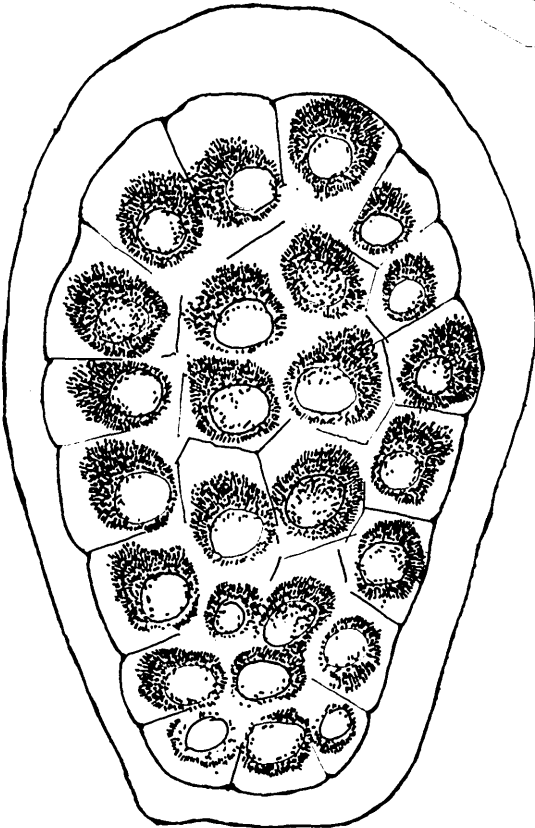
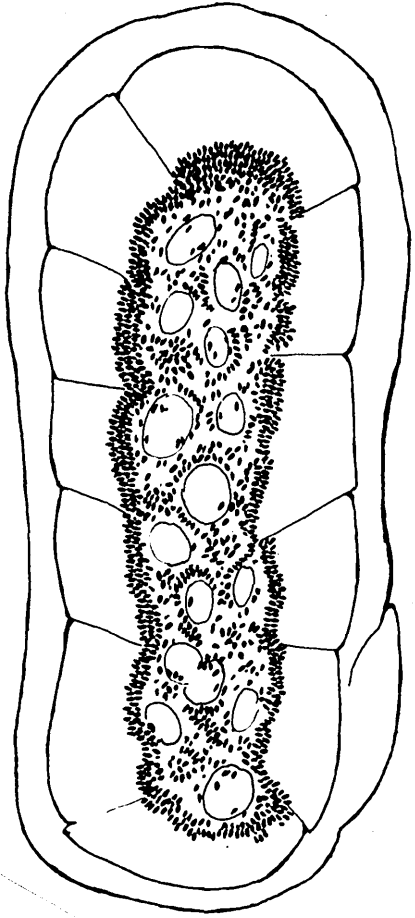
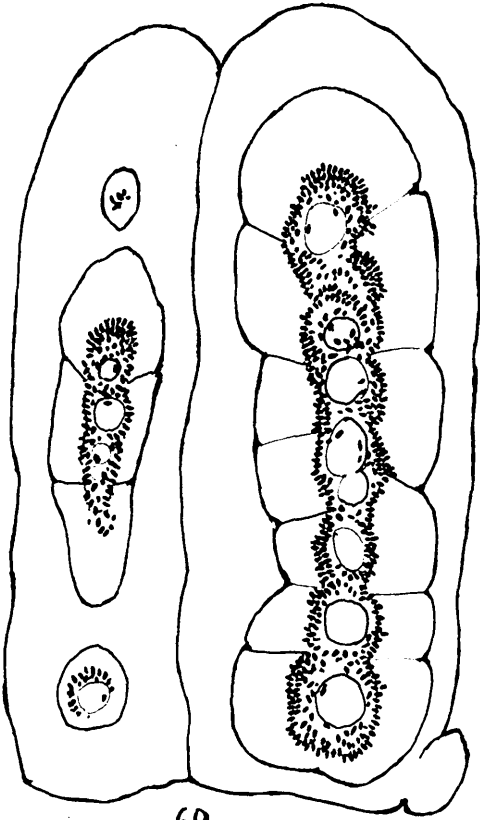
The "Australian Violet".

This very distinct variety is characterized by a bright bluish violet colour of the regions which correspond to the green ones in the wild-type. This colour shows its highest intensity on the rump. It is quite different from any shade possible in "Mauve" birds. Neither single feathers of the "Australian violet" specimens nor portions of a single feather show any such differences in their shade as those seen in "Dark" specimens (p.61). The colour of the "Australian violets" is, therefore, not only brighter but also more equally distributed than that of the "Mauve".

A comparison of a complete feather of the rump of a "Mauve" and of an "Australian violet" specimen shows at a glance that the barbs of the latter are considerably broader. Moreover, in reflected light the surface of the barb of the "Australian violet" shows a cellular mosaic of polygons both more intensely and more uniformly coloured.

From the material examined, it appears that the barbules of the rump of the "Australian violet" bird do not contain the dense and rather regular pigmentation seen in the wild-type (pp.23, 24, Fig.18). Instead, there are only a few irregularly distributed and small but rather dense groups of granules near the terminal ends of the barbules.

Transverse sections of a violet feather of the rump show a very large horizontal diameter. Particularly in the basal portions of the barb, it is larger on an average than in corresponding feathers of the wild-



- 69, 70) Sections through barbs of a feather taken from the rump of an "Australian violet" specimen, in their basal half. Fig. 69 shows a section just above the insertion of barb.
- 71) Section through a barb of a feather taken from the cheek-patch of another "Australian violet" specimen, broadest portion of the barb.

(Fig. 69 - 71: magnification $\times 1300$.)

type (Compare Fig. 69, 70 with Fig. 16). The arrangement of the medullary cells is mostly an irregular one, owing to their great number in the broad section. Only quite close to the insertion of the barb it becomes uniserial (Fig. 69).

The cloudy zone shows the same average width as in the corresponding feathers of the wild-type. The colours reflected and transmitted by this medium, however, resemble those of the "Olive" and "Mauve" specimens as well as those of the cheek-patch of the wild-type: they are violet-blue in reflected, and golden-yellow in transmitted light. The axial pigmentation is complete, consisting of the same type of ovoid granules as in the green feathers of the wild-type.

As stated before (pp. 60, 61), a structural colour produced by the cooperation of a cloudy medium with an absorbing one **becomes** more intense, the greater the width of the cloudy medium, and the more extended the surface of the barbs which are the carriers of both media. Thus, in the "Mauve" type the dull shade is due to the thin cloudy zone and the compressed type of the barb. In the "Australian violet" a much thicker cloudy zone and an extremely broad barb produce a much brighter shade of the same type of colour, somewhat similar to the shade of the cheek-patch of the wild-type.

In the "Australian violet" the width of the barbs does not decrease very much in basal direction. Thus an equally bright shade is produced throughout the region. This may be regarded as a further developmental step towards an intensification of the

colour. Also in this respect, different conditions were found in the "Dark" birds (p.61). A similar intensification of the colour is perhaps caused by the apparently reduced quantity of melanin granules in the barbules of the rump of the "Australian violet".

One of the two specimens examined showed the violet cheek-patch of apparently the same bright colour as that seen in the wild-type, while the other showed a darker and not so intensely coloured mark.

A feather of this region of the second specimen showed some interesting peculiarities. The diameter of the barbs is slightly larger than in the wild-type. In transverse section. (Fig. 71) the cloudy zone shows an average width which is even smaller than in the "Dark" birds (p.62). Moreover, in the wild-type this zone is very regular, while in the "Dark" birds some cells show a somewhat narrower peripheral cloudy portion than the remainder. In some peripheral medullary cells of the feather of the "Australian violet", on the other hand, the pigment corona even reaches the inner wall of the cortex.

The arrangement of the axial pigment in this feather is somewhat different from that in the corresponding feathers of the wild-type (Compare Fig. 71 with Fig. 40). In the feather of the "Australian violet" the bulk of the pigment around each vacuole is not distributed so distinctly peripherally, and does not form such a continuous zone below the cloudy one: the bulk of the pigment of each corona is, of course, directed more ectally.

No specimens are known as yet which combine

the structural peculiarities of the "Australian violet" with the presence of yellow cortical pigment, nor varieties which show a specialization corresponding to the "yellow" group.

The "Cinnamon" Varieties.

The so-called "cinnamon-green" specimens differ from the wild-type by having the underside and the rump of a peculiar dull yellowish green shade which is quite different from that of the "Olive". The dark undulations are of a distinctly brownish colour which has a real cinnamon shade in the juvenile plumage. **At the adult stage**, the barring is mostly much darker than in juvenile feathers, and during the period of moult the contrast is very distinct. Finally, the cheek-patch is much lighter and of a more violet shade than that of the wild-type.

In two articles (1935, 1939), Steiner makes a few remarks on the morphological basis of this peculiar shade: he attributes it to the pigment granules which are "developed in a more or less typical way but show a dark brown shade instead of an intensely black one". Steiner suggests that this difference in the shade might be due to a change of, or deficiency in the **quantity** of the ferment constituent, causing an incomplete oxidation of the tyrosine which is the basic material for the production of melanin.

An examination of sections shows that the colour of the pigment granules is, indeed, much more brownish than in the wild-type. However, the shape of these granules is also quite different: in the green feathers of the wild-type the ovoids are elongated, while those of the "cinnamon" are more or less spherical, and of a diameter equal to about half to two thirds of the length of those in the wild-type (Fig. 73).

In the immediate neighbourhood of the nuclear vacuoles this difference in the shape of the granules is particularly striking; the oblong granules of the wild-type are arranged more or less in a tangential fashion changing **gradually** into a radial position towards the peripheral portions of the axis (Fig. 72). The **quantity** of granules in the axis of the "cinnamon-green" feathers seems to be approximately the same as in the corresponding regions of the wild-type. **In cross section, the axial pigmentation is in these specimens an unbroken ring.**

In the germs of the green feathers of the "cinnamon-green" bird the melanophores are much more transparent than in those of the wild-type. This is due to the smaller size, but apparently not increased number, of the granules. In the wild-type, the melanophores already appear before the formation of the epithelial ridges, whereas in the "cinnamon" they are not visible before the cells in these ridges have arranged themselves in parallel series. In the wild-type a considerable number of contracted melanophores is to be found in the ental residual cell layer, even after formation of the ental parts of the barb (Fig. 7, 8). In the germs of the "cinnamon-green" bird, on the other hand, none could be seen **at this stage.** However, the first stages of formation of the brown modification of the pigment, as well as very small groups of these lighter granules might **escape the attention of the observer more easily than the corresponding stages of the black one.**

In the undulated feathers and in the barbs of the violet cheek-patch the pigment granules are also smaller and of a more irregular shape than the rods in

the corresponding feathers of the wild-type. Among the former, a few rounded dots of about 1μ in diameter may be seen here and there: owing to the lighter brown shade of the pigment, however, it cannot be decided whether these structures are larger granules or only small and closely packed groups of the usual rods.

As in the green barbs, the axial pigment granules of the violet cheek-patch of the "cinnamon" specimens are as regularly distributed as in the corresponding feathers of the wild-type. However, owing to the smaller size of the granules in the former, the pigmentation appears more diluted. As the colour of the pigment itself is not more intensely brown as that often seen in the cheek-patch of the wild-type (see p.44), this diluted state of the pigmentation might be responsible for the shade of the cheek-patch, which is more violet than in the wild-type.

The brown pigment of the "cinnamon" birds is also practically insoluble even in high concentrations of alkali-hydroxide. The dark granules gather on the bottom of the test-tube, and the fluid does not show the slightest brown stain which would be typical for phaeomelanins. *sf*

Kawamura (1935) points out that the shape of the pigment granules found in the epidermis of bird embryos shows close resemblance to that of the mitochondria found within the cells of the same tissue. Moreover, he established transitory stages between the typical colourless mitochondrion and the dark pigment granule. We have seen that the difference in the shape of the granules between the wild- *transitional*

type and the "cinnamon" birds is very distinct and constant. Thus there are more constitutional differences between the two varieties than Steiner assumed (p.85).

Apart from "cinnamon-green" birds which correspond in the distribution of pigment to the wild-type, "cinnamon-yellow" specimens are rather frequent. The latter differ in their appearance from the typical "yellow" ones by the colour of the underside and the rump, which shows hardly any greenish shade and which contrasts little with the yellow of the throat. The cause of this practical absence of the green shade is obviously the reduction of the absorbing medium; the smaller size and lighter shade of the irregularly distributed axial granules renders them much less optically efficient, compared to the state in the typical "yellow". In the undulated feathers we find the same irregular distribution of the medullary pigment as in the "yellow"; owing to their close packing, the dense pigment "balls" (Fig. 51) appear here as dark and opaque as in the typical case. This might be the cause for the resemblance in the shade of the undulations between the "yellow" and the "cinnamon-yellow" birds.

The pigmentation of the "cinnamon" type may occur combined with the "Dark" canalicular structure: in "cinnamon-Olive" birds the green shade is correspondingly much duller than in the "cinnamon-green" bird but lighter than in the typical "Olive" one. (F)

F) "Cinnamon-blue" specimens are mentioned by various breeders. In their particulars they may resemble the "cinnamon-green" ones, except for being alipochromatic.

The "Fallow" Varieties.

Specimens known under the name of "fallow" resemble the lutinos in the eye colour which is persistently "pink" (F). Their underside is of a pure yellow without any green shade, while the rump shows the same yellowish-green colour as in the typical "yellow" birds. The undulation and all parts of the plumage which are black in the wild-type are lighter **or darker brown in different specimens.** Contrary to the case in the "cinnamon" birds (p. 85), this seems to be a mere individual variation, as there exist fully adult specimens with very light brown bars and others with rather dark ones.

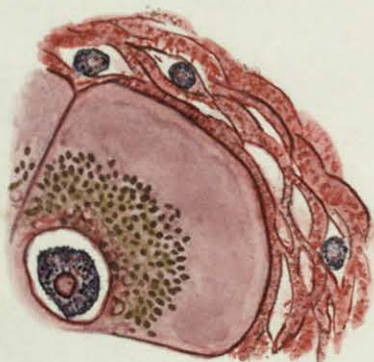
A microscopical examination of a complete feather of the rump in reflected light, shows that the similar shade of this region in this variety and in the "yellow" respectively ~~is~~ brought about in a different way: in the "yellow" bird there is a rather rough pattern of distinct green and yellow spots (p. 50), whereas the "fallow" feather shows a rather uniform shade.

Transverse sections show the reason of this appearance. We have seen that in the feathers of the "yellow" specimens the axial pigmentation is exceedingly incomplete and irregular (Fig. 52). In the feathers of the corresponding region of the "fallow" bird a brown pigment is regularly distributed through-

F) In the nestlings of the "cinnamon" birds the eyes appear also "pink". It is not known whether in both cases the pigmentation of the chorioid membrane is **absent**, as in albinos and lutinos, or whether there a reduced pigmentation is still present.



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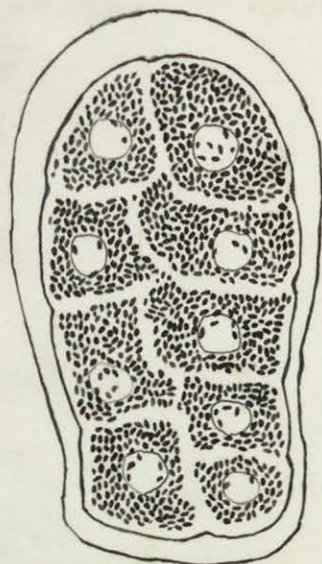


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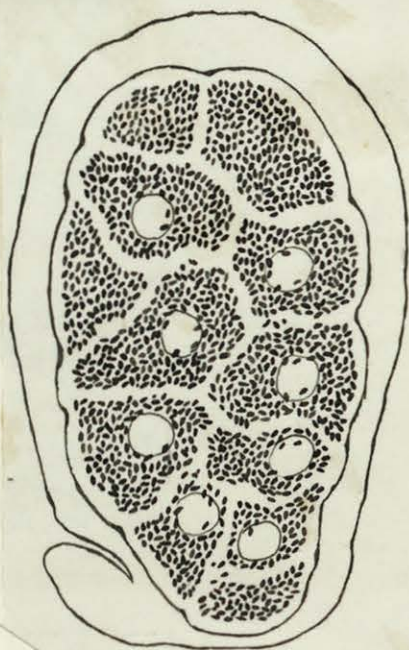


74

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75



76

- 72) Melanin granules in the medullary axis of a feather-germ taken from the abdomen of a wild-type.
- 73) ditto., from an immature "cinnamon-green" specimen.
- 74) ditto., from an adult "fallow (-green)" specimen.
- 75) Section through a barb of a feather taken from the abdomen of a homozygous "Dominant grey" specimen.
- 76) ditto., rump, same specimen.

(Fig. 72 - 74: magnification $\times 1500$,
- 75, 76: - $\times 1300$,

Fig. 72 - 74; - Staining:

Ehrlich's Haematoxyline-
Eosine.)

out the axis of the barb.

Steiner (1935, 1939) mentions that the shape of the pigment granules in this variety is "incomplete". The granules are, of course, very small and have apparently quite irregular outlines (Fig. 74); neither their average sizes nor their shapes can be made out. Partly they are hardly visible between the perinuclear vacuoles while here and there they form dark lines along the margin of the nuclear ones.

In the pure yellow feathers of the underside there is also a complete axial pigmentation of the same type of granules. This axis, however, is narrower in average than that of the barbs of the rump, and this difference is, apparently, the cause of the difference in shade mentioned above.

In the germs of the feathers of the abdomen of a "fallow" specimen there are similar conditions as regards the melanophores as in the "cinnamon-green", except for the very small granules inside these cells, which render the latter even less visible than those in the "cinnamon-green" bird.

The granules found in the barbules of the rump, in the cortex and the axis of the undulated feathers, and, finally, in the axis of the pale but distinctly violet cheek-patch cannot be well distinguished from those in the axis of the barbs of the abdomen and the rump, owing to their exceedingly small size. In the undulated feathers, however, the medullary pigmentation is distinctly axial, and the cortical one too shows exactly the rhythm represented in the diagram Fig. 27.

For this reason, and also because of the complete

axial pigmentation in the feathers of the underside and the rump, and, finally, the absence of large masses of melanophores in the ental portions of the epithelium of the germ, we must consider these specimens, like the "cinnamon-green" birds, as corresponding to the wild-type, and give them therefore the name of "fallow-green". As there has been some doubt whether these specimens ought to be considered in this way or as "fallow-yellows", an explicit statement of the facts appeared necessary.

Apart from these "fallow-green" specimens, feathers of a "fallow-blue" have been examined. This bird shows a very faint suffusion of an ultramarine- or cobalt-blue shade on the abdomen and the same, but more distinct shade on the rump. The undulations are of a darker brown shade than in some "fallow-green" specimens. We may assume that this very slight shade of structural blue on the underside is visible only when the cortex is absolutely colourless, and that the presence of a yellow filter would, in this case, not produce a greenish suffusion, but make this structural shade disappear altogether. Another specimen combines the pigmentation of the "fallow-blue" with the "Dark" structure; owing to this combination the abdomen shows a faint and dull but decidedly lilac suffusion.

Steiner (1939.) explains the characteristic brown shade, as well as the irregular shape of the pigment granules, ~~as due to a change in~~ the basic tyrosine constituent, in contradistinction to his hypothesis made with regard to the "cinnamon" pigmentation

(p. 85). This conception accords well with the relationship established by Kawamura between the mitochondria and the pigment granules (pp. 87, 88), provided the mitochondria, which are apparently of very constant size and shape, carry the tyrosine, while the ferment is added "from outside". In this case a practically equal number of smaller mitochondria would mean a reduced supply of tyrosine.

The brown pigment present in the feathers of "fallow" specimens is as resistant against alkali-hydroxide as that of the "cinnamon" group.

The "Dominant Grey" Varieties.

a) Feathers of a homozygous specimen.

The general appearance of those regions which correspond to the green ones in the wild-type is of a uniform grey which is quite neutral on the under-side. On the rump it is somewhat darker and shows a very slight suffusion of a bluish, but not in the least violet shade.

The fact that these regions are completely uniform and do not show the least mottling, and further the absence of the slightest violet hue distinguish this type at a glance from any "Mauve" specimen.

The undulations are of a deep black, and, under the microscope, the ^{transitional} ~~transitory~~ zones corresponding to the green ones in the wild-type (p.29) turn out to be neutral grey.

The feathers of the rump show, in reflected light, a not very distinct network of broad, light bluish grey strips which surround more or less circular, darker grey spots. These, however, vary in their shade in much the same way as the cellular polygons visible on the surface of a green barb of the wild-type. The barbules of the feathers of the rump show the same rather regular pigmentation as shown in Fig. 18, except that in the examined material of the "Dominant grey" the reduction of the pigment towards the base of each barbule is a more gradual one. The pattern of the surface of the barbs of the abdominal feathers is the same, except that the network is not

as distinctly bluish as in the feathers of the rump.

Transverse sections through these feathers (Fig. 75, 76) show a structure quite different from that seen in ~~any of the~~ other varieties dealt with so far. The outlines of the sections are oval, like those of the rump of the wild-type. The cortex is colourless and does not contain any pigment granules; its ectal thickening is very slight.

The ring-shaped zone corresponding to the cloudy medium in the wild-type is here exceedingly narrow and it communicates with a network of **equally** narrow strips which divide the medullary region ~~into~~ a number of irregular polygons. Only traces of cell-boundaries may be seen here and there in the peripheral parts of the network, along the middle portions of the narrow strips. Each of the polygons mentioned above may show in its internal part a vacuole of the size and shape of the nuclear ones. The remainder of its area is filled out more or less equally with granules of the same shape and size as the axial ones in the green feathers of the wild-type. Here, however, the ~~pos-~~
~~ition~~ of each granule is, except for the tangential one in the immediate neighbourhood of the vacuole, more irregular, and therefore only a few of them are seen in their full length, while the remainder appear **foreshortened**. Here and there within the section of such a polygonal cell, the granules may form a kind of **eddy**, a structure which will be explained later.

The network shows only occasionally, in the area communicating with the peripheral ring-shaped zone, traces of the colours seen in the cloudy medium of the wild-type. Elsewhere it transmits **white** light even

in dry sections (F). Therefore it cannot be considered a cloudy medium proper.

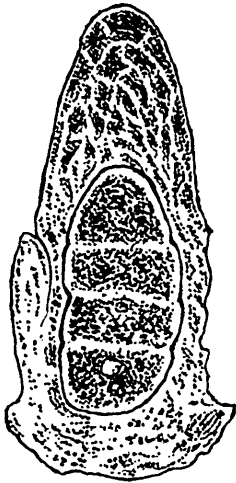
Thus, we find that the formation of a continuous axis by a very close arrangement of the nuclear vacuoles with their pigment coronae in the very centre of the medulla is here abandoned. Of course, the pigment granules are also here arranged more or less regularly around a large vacuole in each cell.

However, these vacuoles occupy each the internal part of the cell to which they belong, being thus more or less regularly distributed over the cortical area of the section. Further, not only the peripheral portion of each medullary cell, but a narrow external zone surrounding completely each of these cells, is pigmentless.

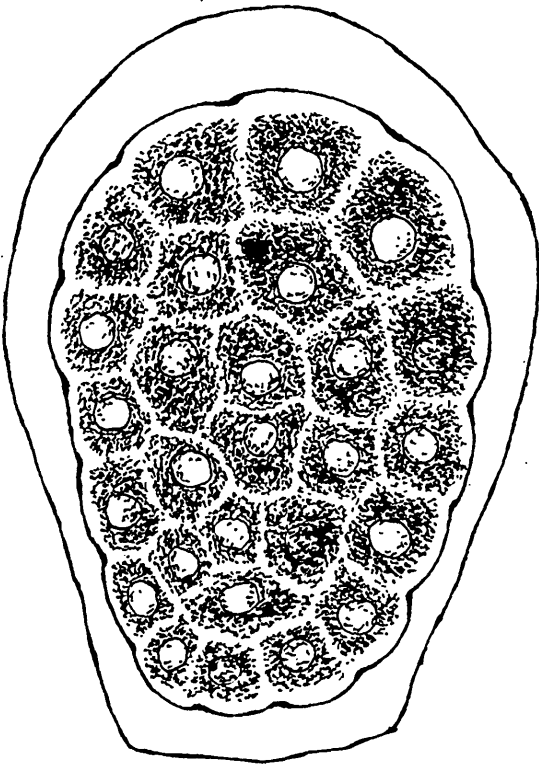
Therefore, the arrangement of the granules in the medulla does not result in the formation of an axis proper, but of discontinuous groups which are regularly distributed, each of them occupying the internal portion of an individual cell.

The same arrangement of the pigmented and the pigmentless portions of the cells can be seen in the dark undulations (Fig. 77), except for the more ladder-shaped pattern formed by the external parts of the cells and caused by their uniserial arrangement. In the neutral grey cheek-patch too the same principle

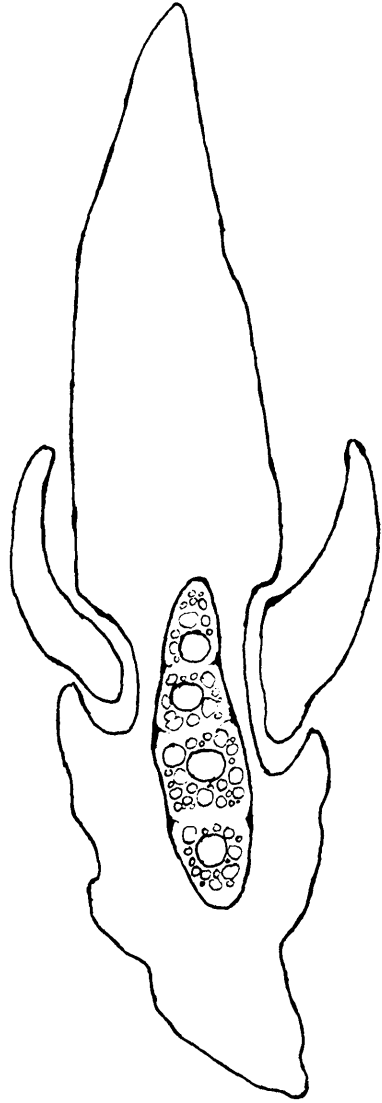
F) In the first sections of these feathers, examined in the course of this investigation, these extremely small portions of the cloudy medium showed a pale buff colour in transmitted light (Auber 1939), owing to the fact that they have not been quite dry. See also pp. 59, 60.



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- 77) Section through a barb of a feather taken from the vertex of ~~the~~ same homozygous "Dominant grey" specimen as in Fig. 75, 76, within a dark transverse bar.
- 78) ditto., cheek-patch, broadest portion of the barb, same specimen.
- 79) ditto., forehead, same specimen.

(Fig. 77 - 79: magnification $\times 1300$.)

has a modifying effect on the structural colour. (Fig. 78).

In the sections of the feathers of the white forehead (Fig. 79) we find that the wall of the medullary cells is comparatively thinner than in the corresponding feathers of the wild-type (Fig. 28). Moreover, there are a great number of apparently perinuclear vacuoles situated between the large central ones and the inner wall of the cortex. Cell-boundaries are hardly visible within the medulla. Thus, we find here an approximation to the type of the thin-walled cells described on pp. 36-40 (Fig. 34, 35).

As, in the other types of feathers of this variety, the pigment more or less covers the structure of the internal parts of the medullary cells, we are in general unable to see any details there. We may, however, conclude that the above-mentioned eddies formed by the granules and found within the cell-bodies probably correspond to larger vacuoles in the cell-body, of the type seen in Fig. 35. Here the knife probably passed just above or below the vacuole. Occasionally a vacuole and one or more eddies can be seen in one and the same cell. Therefore the cavities seen in the sections of barbs of this variety might not all be nuclear ones.

b) Feathers of heterozygous specimens.

Two "Dominant grey" specimens heterozygous for "skyblue" were examined. In one of them, the grey shade is lighter, and it shows, particularly on the

rump, a very intense blue suffusion. The cheek-patch is not as neutral ash-grey as in the homozygous specimen described above. It is, of course, much lighter and of distinctly blue shade without the least trace of violet, thus differing also from the "sky-blue" ancestor. The other specimen seems to be in every respect intermediate between that and the homozygous one.

In the first specimen which shows the more intensely blue shade, the sections of the barbs of the rump (Fig. 80) show in every respect an intermediate state between the extreme homozygous type and the wild-type (F). The outlines of the section seem to be somewhat compressed and, like those of the "Dark" birds, irregularly scalloped, owing to the irregular shape of the medullary cells. The peripheral pigmentless zone of the medulla is very irregular, and its lateral portions in some places much broader than in the homozygous bird. There are, on the other hand, some areas where the pigment granules reach the cortex. Within the broader portions of the pigmentless zone very distinct cell-boundaries can be seen here and there. In reflected and in transmitted light this zone behaves just like the cloudy medium of the

F) Steiner (1935 A) gives a semi-diagrammatic drawing of a section through a barb of a "grey" bird. This drawing is apparently based on such an intermediate feather and not the extreme type seen in the homozygous bird examined here. In this article too, Steiner continues to attribute the pigmented and the pigmentless portions of the medulla to different cells; consequently, in the case of this "grey" barb he speaks of "not entirely missing but extremely flat canalicular cells".

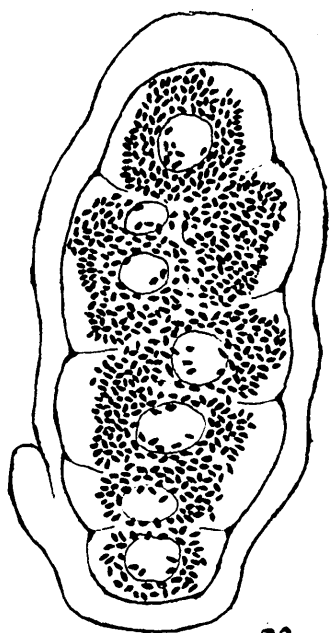
wild-type, in accordance with the intense, blue suffusion of the complete feather.

On the other hand, there are only slight indications of the network found in sections of the barb of the homozygous bird. Thus the arrangement of the pigment granules represents in the heterozygous bird an obvious approximation to the typical axial one. Although the arrangement of the medullary cells is more or less uniserial in these sections, the distribution of the large vacuoles is not very regular. In general, we see here a structure exactly intermediate between the extreme type of "Dominant grey" and the wild-type.

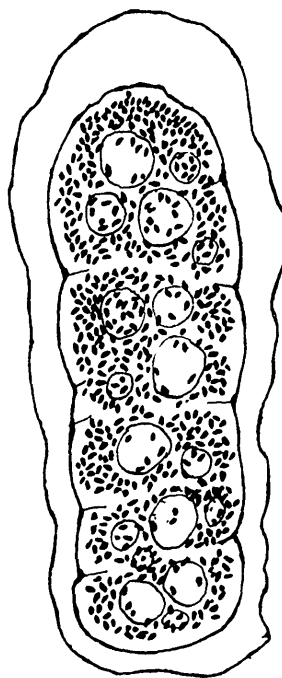
Sections through barbs of the rump of the second specimen (Fig. 81) show again a structure which could be described as intermediate between the above-mentioned heterozygous and the homozygous one. The medulla has a narrower peripheral zone with a less developed cloudy structure, and, on the other hand, more distinct indications of the network.

Sections through a barb of the bluish cheek-patch of the first-mentioned heterozygous specimen show again a state intermediate between the barbs of the homozygous bird (Fig. 78) and the wild-type (Fig. 40), except that the cloudy elements do not show the colours typical of the cheek-patch, viz. violet and yellow, but those seen in green feathers, viz. sky-blue and orange-red.

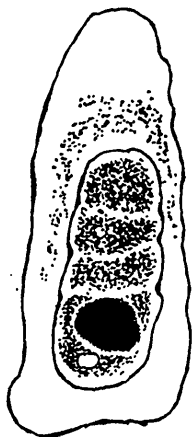
An examination of a germ of the rump of the second specimen shows a closer approximation to the homozygous bird. So far as the dense medullary pigmentation permits of observation, cells similar to



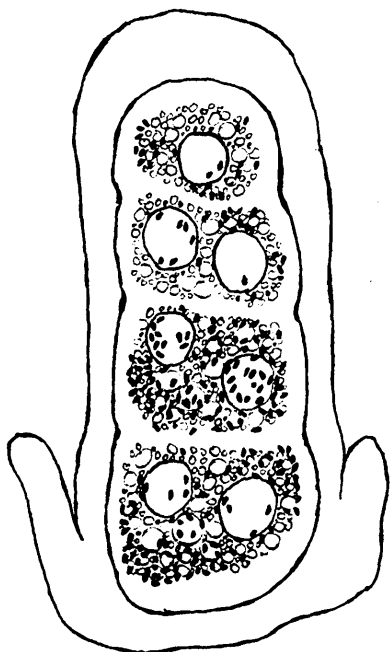
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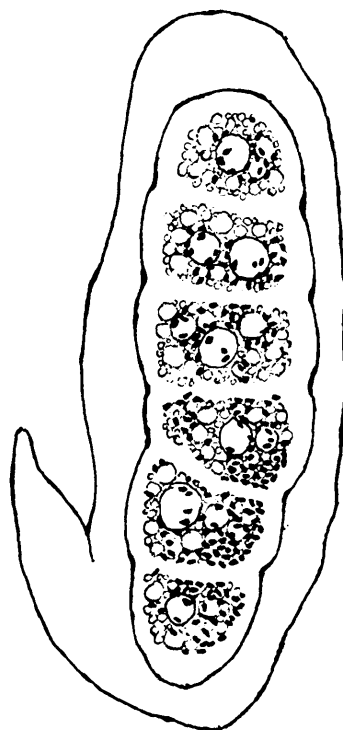
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- 80, 81) Sections through barbs of rump-feathers taken from two different specimens of "Dominant grey", heterozygous for "skyblue".
- 82, 83) *Long rump* ditto., rump of a "white Dominant grey" specimen.
- 84) Same specimen as in Fig. 82, 83: section through the barb of a vertex-feather within a dark transverse bar.

(Fig. 80 - 84: magnification x 1300.)

the thin-walled ones described on pp. 36 ff. appear to be present. Here too the nuclei in the presumptive medulla may lie more or less eccentrically, and, apart from the vacuole formed by the shrinking nucleus, some others of about the same size are found in each cell.

Further, there are no vacuoles in the pigmentless, narrow external parts of the medullary cells. These portions of the cell-body seem to be quite solid. Comparing the typical thick-walled cloudy cells, which contain the restricted zone of perinuclear vacuoles, with cells showing a more extended vacuolization, we find that, in general, pigment granules are fixed only in the framework left between the vacuoles, while the solid or the proper canalicular portions are mostly devoid of granules. In this way, axial arrangement goes together with the restricted zone of perinuclear vacuoles proper, while the more scattered pigmentation of the "Dominant grey" birds is correlated with the vacuoles more widely distributed over the cell-body. Finally, the radial arrangement of the axial granules might be caused by the very thin trabecules between the perinuclear vacuoles which would only permit a lengthwise arrangement of the granules. In the cells of the "Dominant grey" feathers, on the other hand, a more solid framework would also permit a transverse arrangement of the individual granules.

In general, the cell-bodies of the medulla of this variety are smaller than those of the wild-type. This explains the distinctly compressed outline of the sections, seen in Fig. 80, 81 in contrast to Fig. 14, 15 representing the wild-type. Thin-walled cells,

or cells which approximate to this type, apparently undergo earlier keratinization than the canalicular cells, and consequently the growth of the cytoplasm might be sooner inhibited in the former.

c) The "white-Dominant grey" specimen.

A specimen, bred from heterozygous "Dominant grey" birds had on the whole the appearance of "white" birds (pp. 49 ff.). The suffusion on the underside and the rump is, however, of a neutral greyish shade, and, as usual, a little more intense on the rump. The cheek-patch is also pale bluish-grey, again without any violet shade.

Sections of the feathers of the rump (Fig. 82, 83) show the compressed outline and the uniserial arrangement of the medullary cells seen in the heterozygous "Dominant grey" specimens. Owing to the restricted and irregular pigmentation, which corresponds to that of "yellow" birds, "whitesky", etc., the cellular structure is here much better visible than in the "grey" specimens described before. We find a close resemblance to the thin-walled cells described on pp. 36 ff. and represented in Fig. 34, 35 . The formation of vacuoles within these cells seems, however, never to reach such an extreme stage in the "Dominant grey" birds, as in typical thin-walled cells; not even in full-grown feathers of the grey regions of this variety could any quite hollow medullary cell be found. Possibly, an earlier beginning of keratinization in the "grey" feather-germs stops the increase in size of the bubbles sooner than in the typical thin-walled cells, and thus provides a more

resistent framework inside the cell.

The dark bars of the undulated feathers of this specimen (Fig. 84) combine the typical "grey" structure seen in Fig. 77 with elements characteristic of the pigmentation of the "yellow" group. Accordingly we find in these sections dense and well-defined pigment masses scattered among the typical "Dominant grey" pigmentation.

d) "green-Dominant grey" specimens.

At first glance, these specimens look very much like "Olive" ones. A closer examination of their plumage, however, shows that the olive-green shade of the underside and the rump is as uniform as the grey colour of the same regions in the "Dominant grey" birds, while in "Olive" specimens particularly the rump shows a distinct mottling (p. 61). Moreover, the cheek-patch is here distinctly bluish-grey, and not violet (p. 62).

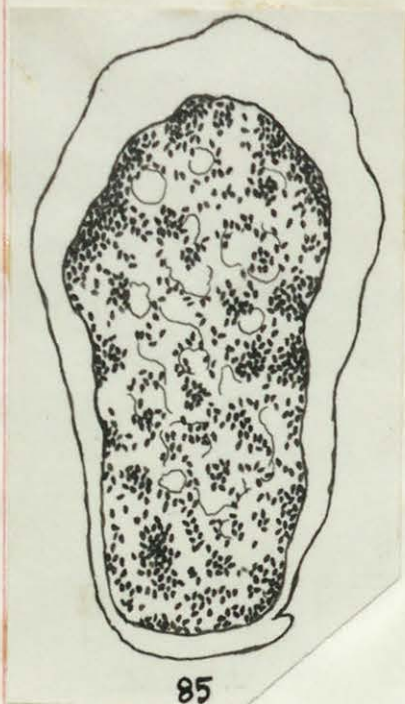
The examination of feathers of one specimen showed, except for the presence of yellow lipochrome in the cortex, a close resemblance to those of the homozygous "Dominant grey" specimen (Fig. 75-78).

e) Conclusions.

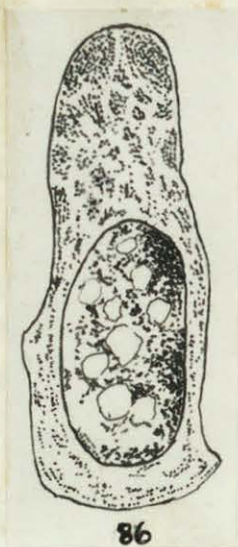
The "Dominant grey" specimens form a very distinct variety which is characterized by the structure of the medulla of the barbs. The cells of this tissue element show, compared with those of the wild-type, a change in the progress of obliteration. As a result of this change they may be

regarded as an intermediate state between the canalicular and the typical thin-walled cells. Correlated with this change is a decentralization of the axial arrangement of the medullary pigment.

The changes affect the whole plumage.



85



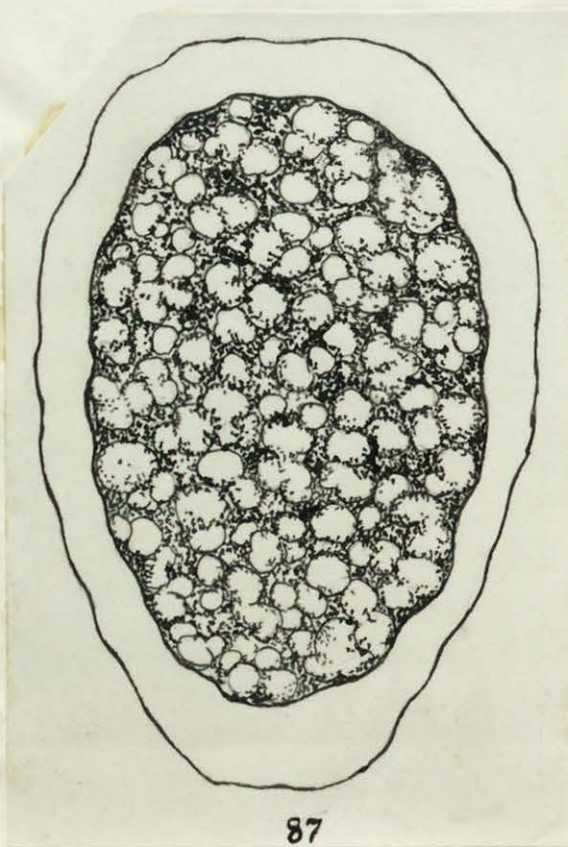
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- 85) Section through a barb of a feather taken from the rump of a "recessive grey" specimen.
- 86) ditto., vertex (dark transverse bar), same specimen.
- 87) ditto., cheek-patch, broadest portion of the barb, same specimen.
- 89) First traces of keratinization in the medulla of a barb in a germ taken from the rump of the same "recessive grey" specimen.
Keratin "spherules" inside the pigment corona (Fig. 88),
ditto., outside the corona (Fig. 89).
- 90) ditto., more advanced stage of keratinization in the same germ: irregular keratin masses have the pigment granules dislocated towards the wall of the cortex which also shows keratinization. In the ectal residual cells, "spherules" are appearing.
- 91) ditto., same germ, terminal portion of a barb: the keratinization is nearly complete, the medulla is filled with a great number of solid keratin masses, partly of ring-shape. Only a few relics of non-keratinized plasma are seen in the medulla.

(Fig. 85 - 91: magnification $\times 1300$,
Fig. 88 - 91, - Fixation: Dubosq,
Staining: Azan.)

The "Recessive Grey" Variety.

a) General characteristics
and intact feathers.

The regions which are green in the wild-type are neutral grey in adult specimens of this variety. This shade becomes, however, darker towards the tip of each feather, thus producing a somewhat thrush-like appearance. This mottled pattern distinguishes the "recessive grey" specimens at once from "Dominant grey" birds (p. 93). The cheek-patch is also dark grey, without any violet or bluish tinge, showing some resemblance to that of the homozygous "Dominant grey".

When examined under the microscope in reflected light, the surface of the barb shows a rather regular polygonal network of very distinct narrow strips of light pearl grey colour, against an intensely black background. Here and there the black gives way to a dark grey shade, thus rendering the lighter strips less distinct. This pattern is quite different from that of the "Dominant grey" bird (p. 93). The barbules of the rump-feathers of the specimens examined show very dense but quite irregularly distributed groups of melanin granules in their extreme tips.

b) Examination of the sections
of full-grown feathers.

The sections of the feathers of the rump are distinctly broader in their ectal portions than in their ental ones (Fig. 85), but they are on the average more compressed than those of the same region of

the wild-type. The cortex shows about the same thickening towards the ectal portions as in the same region of the wild-type and of most of the other varieties. The outlines of both the cortex and the medulla are irregularly scalloped, far more so than those of the "Dark" varieties and the heterozygous "Dominant grey" (p. 97).

The structure ~~within~~ the medulla is not as clear as in other varieties. The distribution of the melanin granules is irregular and in no way correlated to that of vacuoles. Frequently, however, groups of granules reach the inner wall of the cortex. In colour, size and shape, the granules are identical with those found in the axis of the corresponding feathers of the wild-type.

Dry sections do not show in any portion of the medulla colours caused by the reflection or transmission of light through a cloudy medium.

In stained sections, the usual round vacuoles can only rarely be seen. Instead, irregular cavities are frequently found, sometimes of the shape of cracks. It is probable that these cracks and splinters are only artefacts, caused by the extreme brittleness of the keratin. This point will be discussed later on (p. 107).

~~Staining~~ with Gentian violet of feathers of the "recessive grey" gives a result quite different from that in other varieties. In the sections of feathers of, say, the wild-type only the cortical layer takes on the stain, but not the canalicular portions of the medulla. In the "recessive grey" feathers, on the other hand, the cortex remains colourless, while in

the medulla appear some irregular and splinter-like, sometimes even ring-shaped portions, similar to those stained with Azocarmine in the section represented in Fig. 91 .

We have already mentioned that no cloudy medium is visible in dry sections. When we consider the peculiarity in the staining with Gentian violet, we may conclude that in the medulla of the "recessive grey" feathers solid keratin structures are prevalent..

Within the dark transverse bars of the undulated portions, the sections show a similar indistinct structure (Fig. 86) in the medulla, while the cortex and the barbules show the same outline and melanization as are found in the wild-type and other varieties. The shape of the pigment granules in the medulla is the same as in the corresponding feathers of the wild-type.

In the pigmentless feathers, e.g. those of the forehead, no special features of the medullary structure were noted.

Sections of the terminal barbs of the cheek-patch (Fig. 87) show the usual outline. The medulla shows a more regular pattern than in other feathers of this variety. Here too, however, dense groups of pigment granules are situated along the inner wall of the cortex. Within the medulla, on the other hand, the pigment forms a rather regular and continuous network surrounding more or less circular spots which, being stained, are not vacuoles but keratinous structures. Here and there between these stained circular spots and the pigment, small irregular cav-

ities may be seen.

c) Examination of germs of
grey feathers.

Prior to the keratinization of the barbules, the sections show a close resemblance to those of the corresponding feathers of the wild-type. We see, however, a clear differentiation even of the ental portions of the barb, before the beginning of keratinization of the barbules and their withdrawal from the ectal residual tissue (Fig. 7). On the other hand, the flattening of the cortical cells and the typical axial arrangement in the medulla are already very obvious.

At this stage, keratinization begins in a manner peculiar to this variety. The first small round keratinized granules, similar to those seen in the ectal residual tissue of other varieties (p. 21), appear between the nuclear membrane and the axial pigment corona (Fig. 88), sometimes also in the peripheral portions of the medulla (Fig. 89).

Later on, these keratin "spherules" grow in size and fuse with one another, forming more or less **curved irregular masses which** grow apparently by apposition (Fig. 90). In the course of their growth, the keratinizing centres, with originally intra-axial position, dislocate the pigmentary corona towards the inner surface of the cortex. Extra-axial centres of keratinization, on the other hand, may shift the pigment towards the centre of the medulla, during growth.

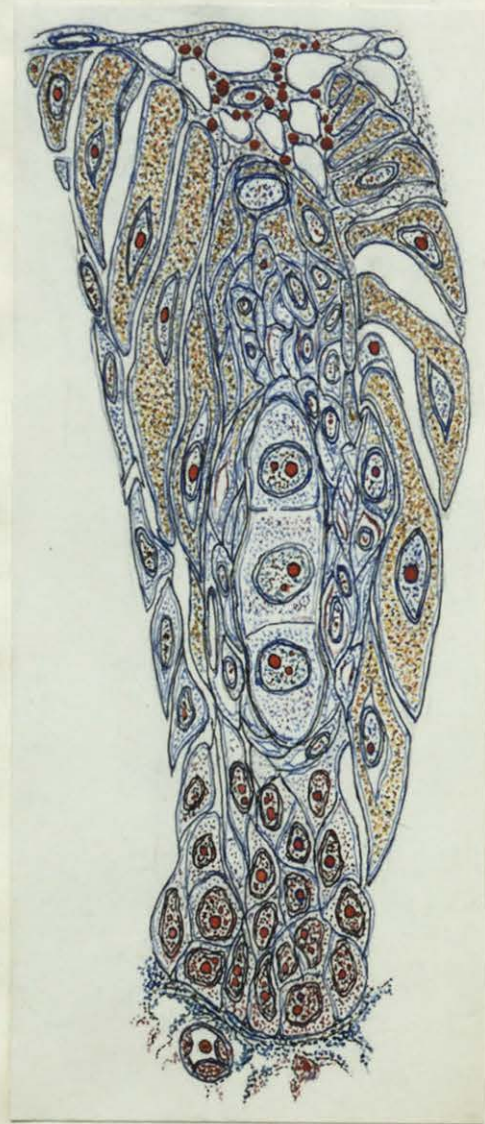
Coincident with the first appearance of these

irregularities in the axial arrangement are the first signs of keratinization in the barbules and the ectal portions of the cortex. They have the same shape as the small refracting cross-sections of fibres, and increase in number in the same gradual way in an ental direction, as described for the wild-type (pp. 19, 20). Together with this keratinization of the barbules and the cortex, the usual "spherules" also appear in the ectal residual tissue, as seen in Fig. 90 .

The irregular keratinization within the future medulla, before the development of the horny framework of the cortex, might be the cause of the extremely irregular and scalloped outlines of the sections of the barbs in this variety (p. 104, Fig. 85).

In the course of keratinization, the irregularly crooked keratinous centres fill more and more of the medullary space. Some of them, gradually surrounding a nuclear space, attain ring-shape, at least in one section (Fig. 91). At the same time, the cortex and the barbules already show the compact keratinous structure of the full-grown feather.

At the stage when the dislocation of the axial arrangement is very advanced, but while large portions of non-keratinized cytoplasm are still visible in the medullary cells, irregular clefts appear here and there in the cytoplasm. **No relics of nuclei were** visible inside these clefts; thus they cannot be nuclear vacuoles. Indeed, they might be artefacts caused by the microtome-knife (see p. 104), or they may have been produced by dislocations in the course of the irregular keratinization of the



92



93

- 92) Same "recessive grey" specimen as in Fig. 85 - 91, feather-germ taken from the cheek-patch. A section is made through the area of a white transverse bar in that part of the germ which develops the basal portion of the feather: only a few "spherules" are to be seen in the ectal residual cells. The most ectal portions of the cortex are in the beginning, the barbules in a more advanced stage of keratinization.
- 93) ditto., same germ, area of the same white bar: the keratinization is in a more advanced stage, a few "spherules" appear in the medulla.

(Fig. 92, 93: magnification $\times 1300$,
Fig. 92, 93, - Fixation: Dubosq,
Staining: Azan.)



- 94) Same feather-germ taken from the cheek-patch of a "recessive grey" specimen as in Fig. 92, 93 (area of the same white bar as in these figures): the keratinization is almost complete. The ental portion of the cortex is well-defined. The medulla is almost filled with spheric keratin masses which have dislocated the nuclei and have left only slight quantities of non-keratinized plasm.

(Magnification: $\times 1300$,
Fixation: Dubosq,
Staining: Azan.)

medulla.

d) Examination of germs of feathers
with compressed barbs.

This type of barb, found for example in the basal portions of the feathers of the cheek-patch, show some other interesting particulars. The barbules show the first "pseudogranular" traces of keratinization and in the ectal residual tissue the first keratin spherules appear in the typical manner, before there are any signs of medullary keratinization (Fig. 92).

Here the first spherical keratin centres appear in the medullary region, before the differentiation and keratinization of the cortex is advanced in ental direction (Fig. 93 , - compare with p. 106).

Further, the number and the size of the keratin "spherules" in the future medulla increases, and consequently the medullary nuclei are pressed into more or less eccentric positions, at first without any deformation of the cell-bodies, so that the originally uniserial arrangement is still undisturbed.

At a later stage, the whole medullary space seems to be filled out by the keratin centres, which, **in contrast to the irregular masses or "rings"** in the grey feathers (Fig. 91), retain more or less their spherical shape (Fig. 94). The nuclei seem to be pressed into the small spaces between the "spherules" and the inner wall of the cortex. At this stage, a number of nuclei may be sometimes seen pressed closely together with very little of the non-

keratinized cytoplasm left. In other sections some of the "spherules" were found pressed together into a polygonal mosaic, without any space left between them.

e) Development of the barbs
of the cheek-patch.

In very advanced stages of keratinization, a great number of "spherules" of almost uniform size can be seen within the medulla of the rigid barbs. Each of these "spherules" is surrounded by melanin granules which are crowded particularly along the inner wall of the cortex, as has been mentioned on p. 105.

Finally, in portions of the germs corresponding to the black bars, the more advanced stages of keratinization show partly "spherules" of rather irregular size, partly **irregular masses within the medullary space.**

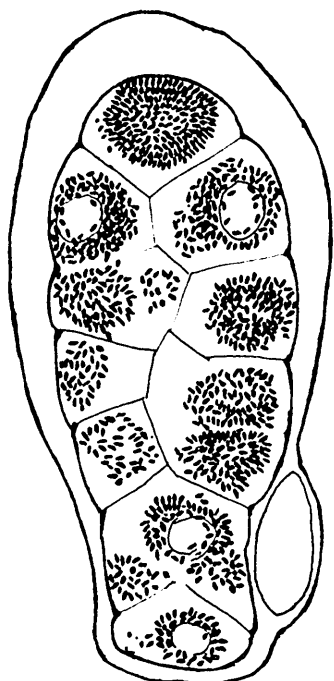
f) Conclusions.

The study of the development of these feathers leads to the explanation of some structural particulars which cannot be elucidated by mere examination of the full-grown feather. We see that here a great quantity of the medullary pigment is not suspended within the keratinous structures, as in other varieties (see p. 99), but is apparently pressed in quite irregular heaps into the clefts remaining between the "spherules", **irregular masses, etc., and the cortex.** As the former structures mostly grow in

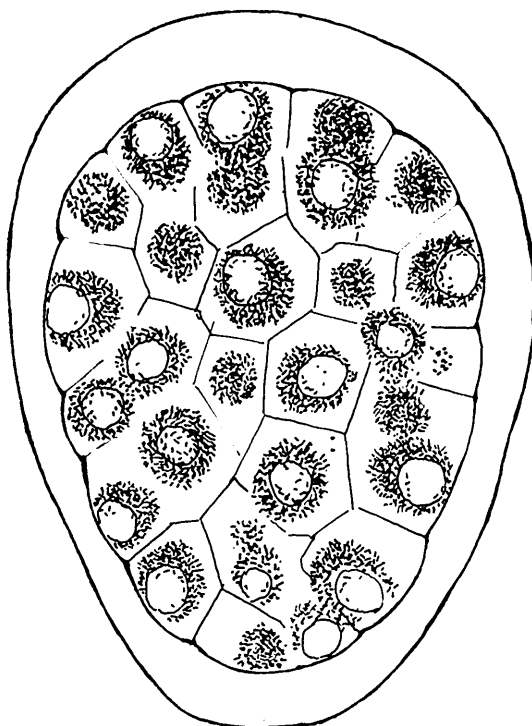
peripheral direction, the presence of pigment immediately beneath the cortex is readily understood.

In short, we may characterize the "recessive grey" as a variety in which the originally typical arrangement of the cells and cell-portions is secondarily disturbed by a factor which effects an early and irregular keratinization within the medulla.

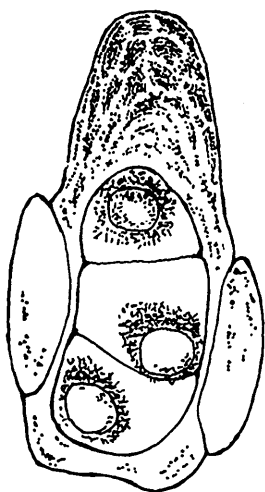
This factor **influences** the whole plumage.



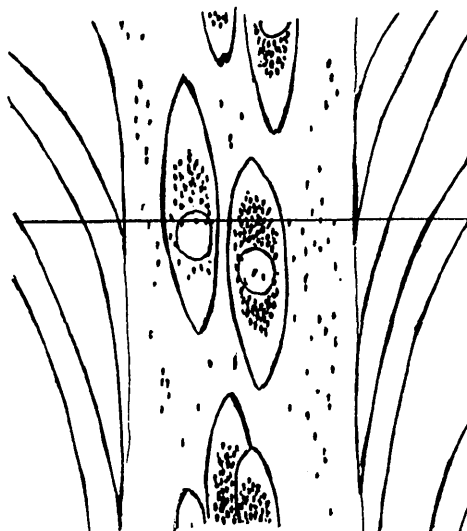
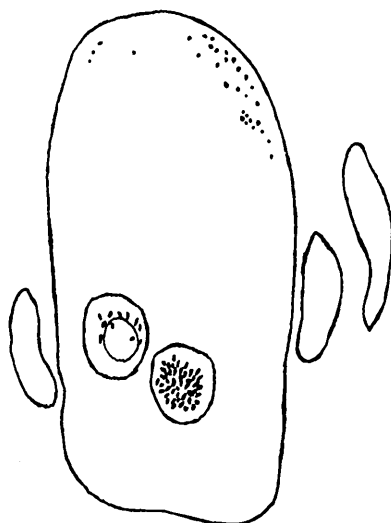
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- 95) Section through a barb of a feather taken from the rump of a "slate" specimen.
- 96) *ditto.*, vertex (dark transverse bar), same specimen.
- 97) *ditto.*, cheek-patch, broadest portion of the barb, same specimen.
- 98) Above: section through a barb of a feather taken from the rump of an immature "silver-blackwing" specimen, showing two medullary cells in section.
Below: general appearance of a portion of another barb in transmitted light, showing the incomplete medulla.

(Fig. 95 - 98: magnification $\times 1300$.)

"Slate" Birds.

Only two "slate-blue" specimens have been examined. These show on the underside and the rump a dull blue shade which, again, does not show the least violet suffusion. As in the "recessive grey" birds (p. 103), these regions show a thrush-like mottling owing to the fact that each feather becomes distinctly darker in terminal direction. The rump is here also darker than the underside. The undulations are distinctly black. The cheek-patch is of a dark dull blue colour without any violet shade.

Steiner (1939) suggested that the peculiar dull shade in these birds might be brought about by the presence of melanin in the cortex as well as in the medulla. The examination of an intact feather in reflected light shows, indeed, many round and indistinct black spots on an intensely blue background. We have seen, on the other hand, that a similar pattern in the "yellow" birds (p. 50) and also in the "recessive grey" ones (p. 103) is caused by medullary pigmentation immediately beneath the cortex. This state is also found in the "slate-blue" variety.

An examination of the sections of the rump (Fig. 95) shows an outline which is considerably broader in its ectal portions than in its ental ones. In contrast to the "recessive grey", the outline is very regular. The cortex, of course, does not contain any pigment. We find, however, a peculiar arrangement in the medulla.

The large vacuoles, each surrounded by a dense pigment corona, are lying close to the inner wall of the cortex. Sometimes the vacuole itself touches the cortex, while in other cases cortex and vacuole are separated by the pigment corona. Between these peripheral vacuoles, more or less large **areas** of unpigmented medullary substance reach the cortex, while communicating in the centre of the medulla, to form there too a large **area**.

A number of continuous cell-boundaries can be observed, extending from the inner outline of the cortex into the central medullary space mentioned above. Examination of dry sections shows that this unpigmented portion of the medulla is of entirely canalicular structure. It shows exactly the same colours in reflected and in transmitted light, as those seen in the corresponding feathers of the wild-type and the "skyblue" birds. In this way a blue, and not violet shade of the feathers is produced, while the dull component is provided by the superficial position of the **isolated** pigment coronae. We may thus characterize this structure as due to an "inversion of the optical media".

The same arrangement may be observed in the medulla of the dark bars of the undulated feathers (Fig. 96), and also in the barbs of the cheek-patch (Fig. 97). Here, however, direct contact between the vacuole and the **cortex is prevalent**. In dry sections of barbs of the cheek-patch, the colours of the cloudy layer correspond to those of the feathers of the rump and the underside in this variety, and not to those of the cheek-patch of the wild-type. The

cheek-patch of the "slate" bird is consequently, as already mentioned, of a neutral blue colour.

The pigment granules in this variety correspond in shape, size, and shade to those of the wild-type.

Examination of feather-germs of the rump shows that, up to the stage when the volume of the medullary cells begins to increase, the development is the same as in the corresponding feathers of the wild-type. From then on, however, it becomes different: not the peripheral portions of the medullary cytoplasm grow out as in the wild-type (p. 16), but the central ones, while the nuclei together with their pigment coronae remain lying close to the future cortex, but lose to some extent contact among themselves.

The factor causing this atypical direction of growth of the medullary cells apparently affects the whole plumage.

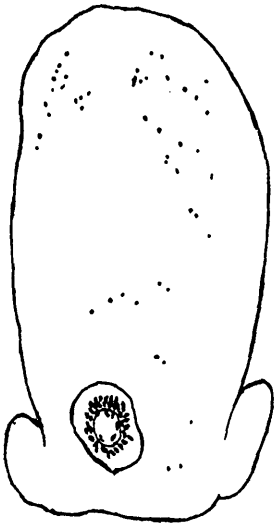
The "Silver-Blackwing" Variety.

The first impression one gets when looking at specimens of this variety is that of a specialization in a way opposite to that of "clearwings" (p. 80). This is also the opinion Steiner (1939) has expressed about this variety.

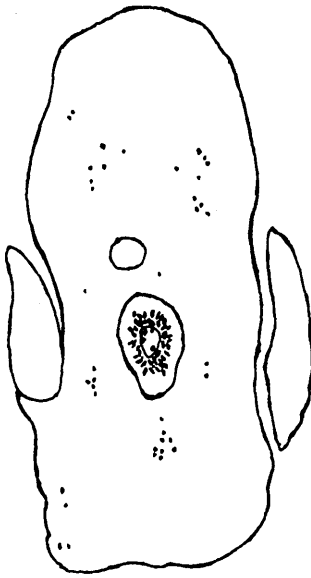
Immature specimens show a "normal" black undulation and black pattern on the quills and rectrices. The rump appears ash-grey, while the underside and the face are of a peculiar silver-white shade which is quite different from the more "milky" appearance in albinos. The absence of the cheek-patch is very striking.

In adult specimens, the grey shade of the rump is to some extent replaced by a dull sky-blue, while a more pure but much paler hue of the same colour more or less replaces the silver-white of the abdomen and the breast. The cheek-patch is indicated by the broader barbs of its feathers which, however, retain their unpigmented state.

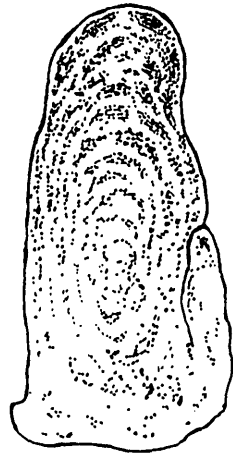
An examination of an intact feather of the rump of the young bird shows in reflected light only a very thin, irregular, and interrupted strip of blue colour along the barb (Fig. 98), while in transmitted light the scattered, spindle-shaped medullary cells show the typical orange-red colour of the cloudy medium in their external portions. Some of these cells show pigment granules in their centre, while in others only a round vacuole can be made out. In the transparent cortex a few pigment granules of ovoid



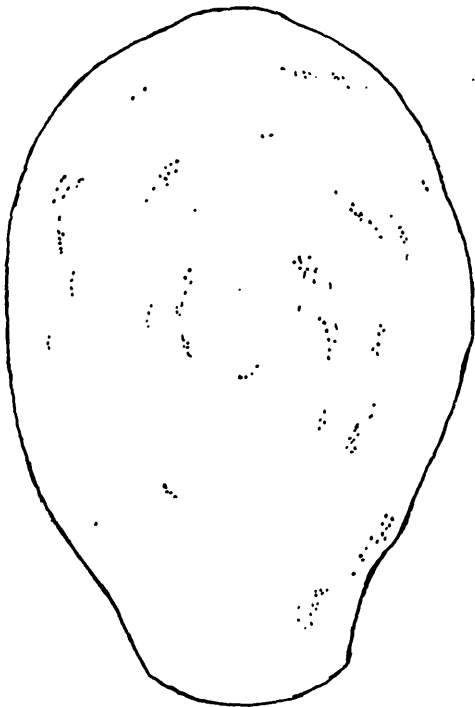
99



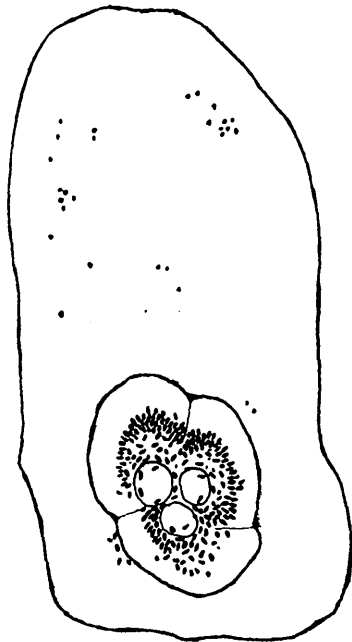
100



101



102



103

- 99, 100) Sections through barbs of the same feather taken from the rump of an immature "silver-blackwing" specimen as in Fig. 98: different positions of the reduced medulla can be seen.
- 101) Section through a barb of a vertex-feather taken from an adult "silver-blackwing" specimen, within a black transverse bar.
- 102) ditto., cheek-patch, broadest portion of the barb, same specimen.
- 103) ditto., rump, same specimen.

(Fig. 99 - 103: magnification x 1300.)

shape can be seen here and there. The barbules show a rather dense but not very regular melanization formed by the usual rod-shaped granules.

In transverse section, these barbs show a rather broad outline (Fig. 99). In some sections no medulla can be seen at all. In others, a single medullary cell of circular outline is present, situated mostly in the ental portion of the section, but sometimes near its centre. (Fig. 100). Again, in other sections, even two cells are present (Fig. 98), separated by a cortical septum.

The silver-white feathers of the abdomen show no medulla at all, and there occur only a few scattered ovoid pigment granules in the cortex. The barbules show, as in all other varieties, no pigment. The silver-white appearance is caused by the lack of the medulla. The latter, containing a considerable quantity of air, would cause the total reflection of a much greater quantity of white light. Here, however, in the absence of the medulla, only the air present between the barbules and the barbs reflects, viz. a much smaller quantity of light, and this might be the cause of the difference in the appearance of white.

In the rump-feathers of adult birds, the medulla is more continuous and shows a larger number of cells in transverse sections. In general, however, the diameter of the medulla is much smaller than in any other variety (Fig. 103). The grey colour, produced in the young bird by the mere effect of the pigmented barbules in cooperation with the practically colourless barbs, is here already replaced by the structural

blue of the barb.

In the abdomen of adult specimens, the juvenile feathers without any medulla are replaced by pale blue feathers with incomplete medulla, as seen in Fig. 98 .

Sections of undulated feathers show the usual compressed outline (Fig. 101). Here too the medulla is completely absent. The cortical pigmentation, on the other hand, corresponds fully to that represented in the diagram Fig. 27 . As the undulation is caused practically only by cortical pigment, it is present here in full intensity and to a full extent.

The quills and tail-feathers show the usual structure of thin-walled cells (Fig. 30) in their ental parts, while the ectal portions show only a very reduced and discontinuous medulla.

The barbs of the cheek-patch (Fig. 102) have in adult specimens the outline and diameter common in the adult birds of all varieties. The barbules are here also completely absent. Owing to the complete lack of medullary cells and the practical absence of pigment, the barbs are quite colourless, but, in contrast to the surrounding feathers, they have a glassy appearance so caused by their large diameter.

In sections of feather-germs of the rump, one finds that the cells forming the central portion of the immensely developed cortex are larger and not as flattened as the peripheral ones and the cortical cells in the corresponding stages of other varieties.

We have already mentioned that, in the "black-wing", the cortical layer even in feathers corresponding to the green ones of the wild-type contains some

irregularly distributed ovoids, which are much more conspicuous than the exceedingly rare pigment granules found in the cortex of the corresponding feathers of other varieties. In the "blackwing" specimens, a number of cells in the centre of the tissue of the future barb have apparently been transformed into solid cortical ones, while the corresponding ones in the wild-type became specialized into medullary cells and form or receive their typical quantity of pigment (see pp. 55, 56). As, therefore, in the "blackwing" the determination of a number of cells has been changed from medullary to cortical ones, the pigment due to them became situated in the cortex, together with these cells.

In basal sections of the germs showing early stages of differentiation, only a small number of not very large melanophores may be seen in the epithelial layer. Other sections, in which the outlines of the barbs are more or less complete, show the number, size and shape of the melanophores in the ental residual layer corresponding approximately to those in germs of the "yellow" birds (Fig. 51). Here too, some branches of pigment-cells extend from the residual tissue into the small but already outlined medulla. However, the dense pigment masses seen in the medulla of the undulated feathers of "yellow" birds are not found here.

We thus see that the specialization typical of this variety is by no means comparable to the merely regional one of the "clearwings". In the "silver-blackwings" it affects the whole plumage, increasing the cortical tissue apparently at the expense of the future medulla.

"Bearded" Specimens.

Some preliminary observations may be recorded here on a very peculiar cause of a colour shade seen in some specimens which resemble "yellow" ones, except for the absence of any green shade on the abdomen. Instead of the throat-spots which are well-defined in "yellow" specimens, an extensive sooty patch with indistinct outlines but of quite symmetrical distribution is found here. Apart from this mark, a faint sooty grey suffusion can be seen on the breast.

Microscopic examination of a complete feather of the throat and of the breast shows an irregular distribution of dark masses between the barbules, which can be washed off with xylol. This fact would lead to the conclusion that these spots and suffusions are mere soilings. However, careful examination showed that the washed off mass consists partly of structures which are very similar to the keratin "spherules" found in the ectal portions of germs and also between the barbules of more advanced stages (Fig. 8). Between these "spherules" many distinct melanin granules could be seen in the isolated mass. In fact, sections of ~~many~~ full-grown feathers showed here and there keratin "spherules" sticking to the concave surface of the barbules, but pigment has never been found there in the course of the present investigations, except in the "bearded" variety.

This form of pigmentation is somewhat reminiscent of the observations made by Greite (1931) on certain varieties of Pigeons.

An examination of germs of this variety, which

so far I have not been able to carry out, may provide an explanation of this unique type of pigmentation.

SUMMARY.

1) Practically all those parts of feathers of the wild-type of the Budgerigar which appear black show pigment granules not only in the cortical layer but also in the axial portion of the medulla. In the latter the pigmentation corresponds to that in green feathers.

2) In the medullary layer of the barbs of green or black feathers of the wild-type each cell consists of a central pigmented area and one or two peripheral canalicular parts. In transverse sections this arrangement provides a continuous absorbing axis surrounded by a continuous ring-shaped cloudy zone. Only the large vacuoles within the pigmented axis are connected with obliterated nuclei.

3) The medullary cells, not only of the green and black but also of the yellow feathers and feather-portions of the wild-type, are all thick-walled and, in their peripheral portions, canalicular. Only the ental portions of the barbs of the quills and rectrices and, **in more restricted manner**, of the wing- and tail-coverts contain medullary cells of the typical thin-walled structure.

4) Owing to the fact that in the undulated feathers of the wild-type no structural difference exists between the black and the yellow bars, no indication of barring is to be observed in specimens in which **melanin** is absent (albinos and lutinos).

? is seen in
as
provides

5) In the Budgerigar the thin-walled cells do not arise simply by a uniform enlargement of the central nuclear vacuoles, for an irregular system of cavities may so expand that the keratinous framework between them breaks down.

6) In the canalicular cells also, small cavities occur apart from the large nuclear one. Even in the wild-type, in those feathers which show functional specialization (see 3) transition stages between canalicular and thin-walled cells are to be found where the keratinous structure is more solid than in typical thin-walled cells.

7) The canalicular structure in the rigid barbs of the violet cheek-patch of the wild-type is different from that in the remainder of the plumage. This modified structure seems to be responsible for the peculiar shade of the patch.

8) In the cheek-patch and also in the throat-spot of the wild-type the shade of the pigment shows some apparently individual variation.

9) In the ontogenetical development of the wild-type the plumage undergoes distinct structural changes on certain regions. These changes render the uniform green regions much brighter at the adult stage.

10) In the feather-germs, the presence of melanin granules has been established not only in the epidermal layer but also in the dermal pulp. Only the former, however, are in connection with the definite pigmentation of the

feather: no evidence could be found of a transference of pigment-cells between these two tissue-layers.

11) In the different colour varieties, in addition to changes affecting the quantity of the pigment, and the canalicular structure and width of the cloudy cell-portions, further changes could be established.

12) The factors causing these changes may have an effect on the development of the plumage which is either

- A) general, or
- B) regional.

13) Changes are to be observed in

- A) the shade and shape of the melanin granules,
- B) the structure and the arrangement of the medullary cells.

14) The melanin with a distinctly brown shade which occurs in the "cinnamon" and "fallow" varieties, as well as in some cases in the cheek-patch of other specimens (see 8), is not a phaeomelanin.

15) In the varieties known under the names of "yellow", "white", and "greywing" the different shades are not entirely dependent upon a quantitative difference in the amount of pigment present in the feathers. In association with the characteristic shape of the pigment cells, the medulla of the undulated feathers shows a

different arrangement of the pigment.

16) In connection with these facts, reasons are given for supposing that the medullary pigmentation in all varieties might originate from the typical melanophores.

17) ~~Confirmation is made of~~ ^{is confirmed,} the conclusion of previous workers, that the pigmentation of the cortex and ~~the~~ barbules may be an autochthonous one.

18) Structural changes may also be controlled by quantitative factors.

These may have an effect on

- A) the quantitative relationship between the cortex and the medulla, so producing
 - a) a thinner cortex: "opaline".
 - b) an extremely thick cortex, with reduction of the medulla: "silver-blackwing".
- B) the development of the canalicular structure,
 - c) with no change in the quantity of the canalicular substance: "Australian violet".
 - d) with reduction of its quantity: "Dark" varieties.
- C) extension of the vacuolar structure in each medullary cell, at the expense of the canalicular one, causing

thus an approximation to the thin-walled type of cells:

"Dominant grey".

D) accelerated and irregular keratinization of the medulla:

"recessive grey".

E) ~~extension~~ of the single medullary cells in an atypical direction during their growth:

"slate".

--oOo--

LIST OF COLOUR SHADES
OF THE VARIETIES.

according to
Ridgway's "Color Standards",
Washington 1912.

- Wild-type, abdomen: yellow green (pl. VI, 31 b-i)
rump (tips): night green (VI, 33 b-i).
- "yellow", abdomen: green yellow (V, 27 b),
rump (tips): viridine yellow (V, 29 b).
- "whitesky", abdomen: beryl blue (VIII, 43 f),
rump (tips): calamine blue (VIII, 43 d).
- "Dark green", abdomen (tips): Peacock green
(VI, 35 i),
rump (tips): meadow green (VI, 35 k).
- "Olive", abdomen: cource green (XVII, 25' i),
rump (tips): forest green (XVII, 29' m).
- "Cobalt", abdomen: squill blue (XX, 45' b),
rump (tips): King's blue (XXII, 47* b).
- "Mauve", abdomen: clear Windsor blue
(XXXV; 49" b-i),
rump (tips): Windsor blue - acetin blue
(XXXV, 49" i - k);
both regions may show suffusions with
shades of Payne's grey (XLIX).
- "lutino", abdomen and rump:
citron yellow - strontian yellow
(XVI, 23' b - beneath b).
- "Australian violet",
abdomen: greyish violaceous blue
(XXII, 51* i),
rump: dull violaceous blue
(XXII, 51* above i).
- "cinnamon green",
abdomen: lumière green (XVII, 29' b),
rump (tips): Scheele's green (VI, 33 i).
- "fallow green",
abdomen: light greenish yellow (V, 25 b),
rump (tips): viridine yellow (V, 29 b).

"Dominant grey" (homozygous),

abdomen: deep Gull grey
(LIII, Carbon grey b),
rump: deep green-blue grey
(XLVIII, 45" b).

"recessive grey",

abdomen (tips): slate grey
(LIII, Carbon grey i),
rump (tips): ditto., somewhat darker.

"slate",

abdomen (tips): Delft blue
(XLII, 45" l),
rump (tips): Delft blue - deep Delft
blue (XLII, 45" i - k).

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- 8) As in Fig. 6 and 7, but more advanced stage. 21

The barbs and barbules are completely keratinized and the nuclei of the medulla already obliterated, the nucleoli still to be seen in the vacuoles. In the cloudy layer two periaxial vacuoles are to be seen.

The ectal residual cells are nearly fully keratinized and form a dense mass of spheric granules, while the ental ones **are separated** from the barb by a keratinized membrane formed of flattened cells.

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- 12) ditto., feather of another specimen, middle part of barb. 23
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- 14, 15) ditto., middle part of barb. 23
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- 22) ditto., another feather of the same specimen: black bar following the most terminal yellow one. 25
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